MINERALIZATION OF ORGANIC PHOSPHORUS IN A SUBTROPICAL FRESHWATER WETLAND

By

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This dissertation is dedicated to the memory of my beloved father.

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS iii

ABSTRACT vi

CHAPTERS

1 INTRODUCTION 1

Overview of the Problem. 10
Objectives and Hypotheses 14
Dissertation Format. 16

2 INFLUENCE OF SOIL FACTORS ON ORGANIC PHOSPHORUS MINERALIZATION ALONG A EUTROPHIC GRADIENT IN THE FLORIDA EVERGLADES 17

Introduction 17
Materials and Methods 19
Results and Discussion 28
Conclusions 54

3 ROLE OF INORGANIC ELECTRON ACCEPTORS ON ORGANIC PHOSPHORUS MINERALIZATION IN DETRITUS AND SOILS OF THE FLORIDA EVERGLADES 56

Introduction 56
Materials and Methods 59
Results and Discussion 68
Conclusions 84

4 INFLUENCE OF PHOSPHORUS-HYDROLYZING ENZYMES AND SUBSTRATE QUALITY ON ORGANIC PHOSPHORUS MINERALIZATION IN FRESHWATER WETLANDS 86

Introduction 86
Materials and Methods 88
Results. 99
Discussion 117
Conclusions 123

5 SUMMARY AND CONCLUSIONS 126

LITERATURE CITED 131

BIOGRAPHICAL SKETCH 147

Abstract of Dissertation Presented to the Graduate School
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MINERALIZATION OF ORGANIC PHOSPHORUS IN A SUBTROPICAL FRESHWATER WETLAND

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A major portion of the phosphorus (P) added to a wetland is stored in the detritus and soil organic matter. In addition to external loading, the decomposition of detritus and soil organic matter and subsequent release of inorganic nutrients regulate wetland productivity and water quality. Hydrological changes and nutrient (especially P) loading to the Florida Everglades Water Conservation Area 2A (WCA-2A) had resulted in invasion of cattail (*Typha* spp.) into formerly sawgrass (*Cladium jamaicense* Crantz) and slough communities and accumulation of P in soil organic pools. The objectives of the study were to determine: (1) the influence of soil factors regulating organic P (P_o) mineralization, (2) the influence of electron acceptors on P_o mineralization, and (3) the effects of phosphorus-hydrolyzing enzymes and nature of the substrate on P_o mineralization.

Organic P mineralization rates were measured for detritus and soils collected along the P gradient in WCA-2A. Substrate-induced P mineralization (SIPM), as a measure of ambient microbial/enzyme activities, had higher rates for the non-impacted interior areas than for impacted areas located near the inflow. Soils had higher SIPM rates under aerobic than anaerobic conditions, while the type of electron acceptor (O_2, NO_3^-, SO_4^{-2}, HCO_3^-) did not affect SIPM rates of detritus. Potentially mineralizable-P (PMP) that provided estimates of the turnover rates for various P_o pools revealed an immediate immobilization of the P released in detritus and no difference among electron acceptors in soils.

Mineralization of P_o was predominantly regulated by alkaline phosphatase (phosphomonoesterase) activities. Phosphorus enrichment, microbial biomass, labile P_o content, and substrate quality (i.e., cattail vs. sawgrass) influenced phosphatase hydrolyzable-P_o. Among the four P_o sources investigated, ATP and glucose-6-phosphate had appreciable hydrolysis, and hydrolysis of phytic acid (more stable) increased with P enrichment.

Phosphorus enrichment increased the microbial biomass P and labile P_o pools; consequently increasing the rates of P_o mineralization in detritus and soil organic matter. Short-term dry periods increased labile P_o mineralization in wetlands. Mineralization rates of P_o and the subsequent release of inorganic P to the overlying water column potentially can regulate the time required in restoring a wetland to a desired condition when P loading has been abated.

CHAPTER I
INTRODUCTION

The dynamic nature of soil organic phosphorus (P_o) is concealed by the concurrence of mineralization and immobilization processes, and the small size of the biologically active pool, in soil organic matter (SOM) (Stewart and Tiessen, 1987). Organic P mineralization is crucial to internal P cycling, and is regulated by the relative proportion of labile and non-labile pools (Fig. 1.1). When plants senesce, a substantial portion of P in the aboveground tissue is translocated into belowground biomass (Davis, 1991). Detrital plant tissue deposited on the soil surface will release P back into the labile pool through microbial-mediated enzyme hydrolysis, while the recalcitrant tissue becomes part of the soil organic matter. The balance between the conversion of P_o to inorganic P (P_i) (mineralization) and the assimilation of P_i into microbial biomass (immobilization) depends on substrate quality (i.e., the $C:P_o$ ratio of the organic matter) and the type of electron acceptors involved in the decomposition (Reddy and D'Angelo, 1994). Net mineralization of plant residues will occur at a $C:P_o$ ratio of less than 200:1, while net immobilization will occur at a $C:P_o$ ratio of greater than 300 (Stevenson, 1986). Mineralization of P_o can occur either through biological or biochemical processes. Rates of microbial growth and respiration control biological mineralization, while the biochemical mineralization which involves the hydrolytic action of extracellular

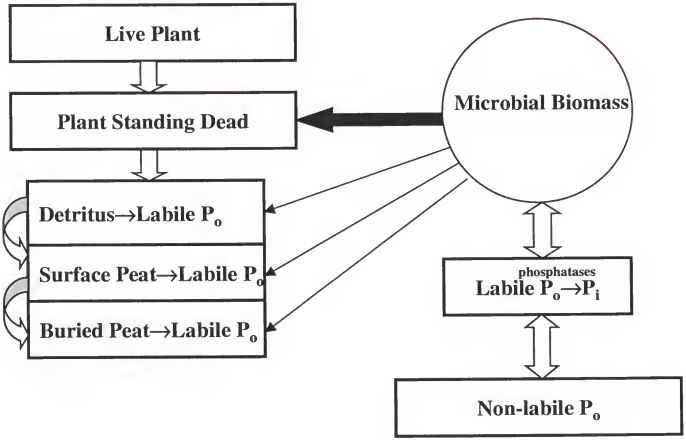

Fig. 1.1. Schematic diagram of organic P (P_o) mineralization in wetlands.

enzymes is driven by the supply of and need for P (McGill and Cole, 1981).

The high rate of primary productivity and slow rate of decomposition in wetlands often lead to accumulation of organic matter, which can provide long-term nutrient storage (Reddy et al., 1993). The rate of organic matter accumulation and its associated nutrients in the soil depends on the quantity of organic inputs (mainly as plant residues), rates of biological decomposition processes, and the ability of soil to protect organic matter from microbial degradation (Duxbury et al., 1989).

A significant portion of nutrients in peat-laden wetlands is stored in live and detrital plant tissue, microbial biomass, and soil organic matter (Davis, 1982; Richardson and Marshall, 1986; Verhoeven and Arts, 1987; Reddy et al., 1993; DeBusk et al., 1994). For example, in the Everglades Water Conservation Area-2A (WCA-2A) more than 50% of the added P is incorporated into the soil and between 12 and 30% is incorporated into plant detritus (Davis, 1982). When plants and microorganisms die their remains are broken down into small fragments until unrecognizable, and the complex organic molecules that made up these organisms are broken down into simpler compounds such as carbon dioxide and water, and soluble nutrients such as nitrogen (N) and P.

Phosphorus is often the key nutrient that limits productivity in estuaries and most freshwater ecosystems (Golterman, 1973; Boström et al., 1982; Vymazal, 1995). However, excess P from the continuous application of P-rich sources to uplands can move into adjacent wetlands and finally into water bodies. Widespread use of P to improve soil fertility is one factor responsible for the global eutrophication of freshwater systems (Tiessen, 1995). Organic waste application that is based solely on the N requirement of growing crops tends to overestimate P such that excess P finds its way

into aquatic systems through wetlands (Sharpley, 1999). Since wetlands form a critical interface between uplands and water bodies, much focus has been given to their role in alleviating the eutrophication problem for receiving water bodies. However, wetlands are by themselves affected by eutrophication, such as in the Florida Everglades.

Unlike carbon (C) and N, P has no gaseous component and, therefore, remains in the system. About 10 to 70% can be retained in organic forms (Sommers et al., 1972). However, nearly half of the P_o that exists in soil still remains to be identified chemically. The P_o concentration in soils is usually estimated based on the difference of total and inorganic P. As with P_i, P_o forms are grouped according to biological availability, chemical extractability, and physical separation (Sommers et al. 1972; Newman and Robinson, 1999).

Organic P is generally greater than 50% of the total soil P in wetlands, while it is highly variable ranging from 10 to 90% of the total soil P in uplands (Hesse, 1962; Syers et al., 1973; Anderson, 1980; Condron et al., 1985). The type of soil, nature of organic P loading from external sources, and deposition of dead algal cells and detrital tissue of aquatic macrophytes can determine the proportion and nature of P_o in wetlands.

Nature of Organic Soil P

Soil P_o is derived largely from plant residues, though some is synthesized from inorganic sources by soil microorganisms (Sanyal and De Datta, 1991). Organic P can be grouped into (1) inositol phosphates, (2) nucleic acids, and (3) phospholipids (Anderson, 1980). The inositol phosphates (hexa- and pentaphosphates) are of plant origin and comprise up to 60% of soil organic P (Tate, 1984). Inositiol hexaphosphate (IHP) forms a major storage compound, particularly in seeds (Wetzel, 1999). Being a structural

component of nucleic acids, coenzymes, phosphoproteins, and phospholipids, P is an important component of most metabolic pathways. The phospholipid content in soils can vary from 0.5 to 7% of total soil organic P (Cosgrove, 1977; Dalal, 1977). Plant debris, animal wastes, and microbial biomass are among the contributors to soil phospholipids. Adenosyl triphosphate (ATP) is a cell constituent that functions in energy storage and transfer, and has been widely used to measure the microbial biomass content of soils. The coenzymes - nicotinamide adenine dinucleotide (NAD) and nicotinamide dinucleotide phosphate (NADP) - are P_o compounds involved in biological oxidation.

While P_o in surface soils occurs predominantly as phosphomonoesters, phosphodiesters that consist of nucleic acids and phospholipids are more common in living organisms (Condron et. al, 1985, 1990; Bedrock et al., 1994; Gressel et al., 1996). Phosphodiesters and complex monoesters such as phospholipids that are resistant to hydrolysis are the primary constituents of phosphatase-hydrolyzable P_o in forest Spodosols (Fox and Comerford, 1992). In wetland soils, orthophosphate diesters can contribute to the bioavailable P pool (Robinson et al., 1998). Differential stabilization between these phosphomono- and phosphodiester forms in soils exists because phosphodiesters are less strongly adsorbed to the soil matrix than phosphomonoesters and, therefore, are easily attacked by phosphatases and recycled immediately (Hawkes et al., 1984; Hinedi et al., 1988). Using ^{31}P-NMR, Condron et al. (1985) observed that more than 90% of P_o in upland soil was in monoester form, while Dai et al. (1996) determined that as much as 39% of the total P in the Bh horizon of a forested Spodosol consisted of phosphodiester. Similarly, Bedrock et al. (1994) measured significant amounts of

phosphodiesters (21-26% of total P) in humic acid of peat soils through ^{31}P-NMR, while no phosphodiester was measured in the humic acid fraction of a mineral soil.

Aerobic-Anaerobic Interfaces in Wetlands

Aerobic and anaerobic conditions arise from the intermittent flooding and draining cycles of wetlands. The balance between these conditions is determined by the supply and demand for oxygen. Increases in oxygen consumption arise from the abundance of carbon substrates available as electron donors in wetlands.

Presence or absence of oxygen can result in three distinct layers formed within the soil. They are: (i) an aerobic soil layer where oxygen is always present, (ii) an alternating aerobic or anaerobic zone, and (iii) an anaerobic layer where oxygen is virtually absent. The thickness of the aerobic layer is dependent upon the net oxygen consumption rate of the soil, and the diffusive and advective movement of oxygen from the water column (Reddy and D'Angelo, 1994). It ranges from a few millimeters to 2 cm in thickness (Patrick and Delaune, 1972). The thickness of the oxygenated layer is influenced by the presence of an active benthic algal community. The photosynthetic activity of periphyton at the soil surface causes an increase in thickness during periods of high photosynthetic activity, when more dissolved oxygen is released to the system (Vymazal, 1995).

During microbial respiration various organic compounds act as electron donors and are coupled with inorganic electron acceptors. Electron donors are usually non-limiting in wetlands. When soils are flooded oxygen diffusion to soil becomes restricted, thereby shifting the microbial communities from aerobic to anaerobic. Inorganic constituents that act as an electron acceptor are reduced preferentially following the sequence: $O_2 > NO_3^- > Mn^{+4} > Fe^{+3} > SO_4^{-2} > HCO_3^-$. This order of electron acceptor

consumption is based on the thermodynamic theory - the higher the reduction potential, the more energy would be available subsequently for microbial metabolism. Several redox reactions, however, can proceed simultaneously due to spatial variability during aeration of soil aggregates, particularly in micropores (Smith and Arah, 1986). The greater amounts of energy released from aerobic compared to anaerobic microbial decomposition suggests a higher rate of organic matter decomposition under aerobic than anaerobic conditions. DeBusk and Reddy (1998) obtained a three-fold increase in potential C mineralization when detritus and peat soils of the Florida Everglades were incubated under aerobic compared to anaerobic conditions. Similarly, McLatchey and Reddy (1998) observed that the hydrolysis rate of added ribonucleic acid P (RNA-P) in wetland organic soils was three times greater under aerobic than anaerobic conditions.

Soil Microbial P

The role of microorganisms in transforming organic to inorganic P has long been recognized. Soil microbes can act both as catalyst and as sources/sinks for nutrient transformations, and are usually referred to as the "driving wheel" of the P cycle. Microbial biomass also can serve as a temporary storage for nutrients. In a bacterial cell, P comprises as much as 3% of the dry weight (Fenchel and Blackburn, 1979). However, microbial biomass is relatively labile and evidences rapid turnover even though it is a small fraction of the organic matter (Jenkinson and Ladd, 1981).

The size and diversity of microbial communities depend on the quality and quantity of available substrates in wetlands (Reddy et al., 1999). Indices of microbial diversity have received limited attention because of the scarcity of detailed information on microbial species composition in soils (Turco et al., 1994). Knowledge of the

microbial diversity, however, can provide an indication of community stability and impacts of environmental stress. Microorganisms influence the rate of P_o mineralization because of: (i) their ability to produce extracellular or periplasmic hydrolases; and (ii) their influence in changing ionic composition, redox, pH, and other conditions that affect the tenacity by which P is bound to organic matter (Wetzel, 1999).

Phosphatases

The high molecular weight and polymeric structure of organic matter inhibit its direct entry into cells. The hydrolysis of such polymers becomes the rate-limiting step in the utilization of organic matter by microorganisms. Various enzymes are then needed for these polymers to be broken down into monomers before they can be incorporated into cells. These enzymes are located on the cell surface or in the periplasmic space (ectoenzymes), and may be particle-bound or released from the microorganisms as free or dissolved enzymes (extracellular) (Burns, 1982). Enzyme assays are generally used as measures of microbial activity but no single assay can best describe the suite of processes collectively known as microbial activity (Sinsabaugh, 1994)

The ability of soil microorganisms to scavenge for nutrients is dependent upon the activities of their extracellular enzymes. These enzymes, however, can be inactivated by denaturation and adsorption or degraded by proteolytic enzymes (Burns, 1978). The survival of soil microorganisms can be threatened in nutrient-limited environments where there is a discontinuity of substrates, both spatially and temporally.

Extracellular enzymes such as phosphatases can regulate the rate of P_o mineralization in wetlands (Freeman et al., 1996; Amador et al., 1997; McLatchey and Reddy, 1998). Phosphatases are groups of enzymes that catalyze the hydrolysis of both

esters and anhydrides of phosphoric acid. These enzymes are classified into five major groups including phosphoric monoester hydrolases, phosphoric diester hydrolases, triphosphoric monoester hydrolases, enzymes acting on phosphoryl-containing anhydrides, and phosphoamidases or other enzymes acting on P-N bonds (Eivazi and Tabatabai, 1977). It was demonstrated in uplands that high phosphatase activity results in rapid turnover of P_o (Halm et al., 1972; Sharpley, 1985). Because of their specificity, acid phosphatases are predominant in acid soils and alkaline phosphatases in alkaline soils. Alkaline phosphatase is a dimeric molecule consisting of two subunits interacting through negative cooperativity (Chróst, 1991). Half of the molecule shows reactivity at physiological pH while the other half conforms to Michaelis-Menten kinetics at alkaline pH. Alkaline phosphatases catalyze the hydrolysis of phosphate esters of primary and secondary alcohols, sugar alcohols, cyclic and phenolic alcohols, and amines to inorganic P. These enzymes also catalyze the hydrolysis of polyphosphates but not of phosphodiesters. They are produced under P-limiting conditions but repressed when orthophosphate-P is above a critical level. The production of phosphatase enzymes is stimulated by the presence of organic matter and P_o (Speir and Ross, 1978). Amador et al. (1997) measured phosphatase activity at ambient pH for riparian forest soils that were moderately well to poorly drained, and obtained a positive correlation ($P< 0.001$) with organic matter and moisture. Soil pH and P_i content (up to 47 mg kg^{-1}) were likewise positively correlated ($P< 0.001$), though for moderately well-drained soils only (Amador et al., 1997). Harrison (1983) observed a positive correlation of phosphatase activity with P_i content up to 50 mg P kg^{-1}. Thus, soil P_i content of less than 50 mg P kg^{-1} is not inhibitory to phosphatase activity. In Lake Pluβsee, the specific activity of this enzyme

decreased significantly when P_i concentrations were above 10 μg PO_4^{-3}-P per L (Chróst and Overbeck, 1987). Strong competitive inhibition of alkaline phosphatase activity (APA) has also been shown to occur when there are high intracellular levels of both ortho- and polyphosphates (Chróst, 1991). Addition of readily utilizable substrates such as glucose, cellobiose, and amino acids was shown to deregulate bacterial synthesis of alkaline phosphatase (Chróst at al., 1989). Values of APA reported for field-moist soils range from 11 to 1000 mg *p*-nitrophenol kg^{-1} h^{-1}, with mean value of 122 (Dick et al., 1996).

Overview of the Problem

Much attention had been focused on the ability of wetlands to transform and remove pollutants rather than on the ecological impacts of the removal process. The need for protection of wetlands at the same level afforded to other water bodies has been recognized only recently (USEPA, 1990). Wetlands vary in their capacities to transport and transform nutrients or pollutants due to differences in physical, chemical, and biological soil properties. Criteria on these soil physicochemical and biological features that would enable wetland scientists to make sound decisions concerning wetland management, creation, and restoration is necessary but lacking (Reddy et al., 1996).

From 1780 to 1980, total wetland area in the continental United States and Florida declined to about 53 and 46%, respectively (Dahl, 1990). Drainage of some wetlands had promoted the oxidation of organic matter and the release of nutrients, particularly P, into adjacent wetlands or water bodies. Aside from oxidation of organic matter, runoff from agricultural lands under long-term P fertilizer application, organic wastes application that include dairy and industrial wastes, and domestic wastes are among the contributors of

external P load to wetlands. Phosphorus loading to wetlands has been reported to affect both the physiological and ecological processes of macrophytes from individual to community level (Miao and DeBusk, 1999). Increase in the overall productivity of wetlands and other aquatic systems can lead to reductions in dissolved oxygen levels.

Pathways of nutrient movement and transformation between living and nonliving components of a wetland can determine the impact of an external nutrient load on a wetland (DeBusk et al., 1994). Among these pathways is the mineralization of organic P, which is regulated by both biotic and abiotic factors. While the bulk of the soil P in wetlands is in organic form, there is currently limited information on the dynamics that affect overall P cycling. Predicting P transformations and potential transfer from agricultural sites to other ecosystems requires not only knowledge regarding the transformation of inorganic forms, but also of organic forms as well.

The Florida Everglades is currently being examined with respect to its historic and present situation. The Everglades is a freshwater ecosystem that evolved under low-P concentrations (Belanger et al., 1989; SFWMD, 1992). Previously, nutrient loading was mainly through rainfall that had typical total P (TP) concentrations of less than 10 μg P L^{-1}, and total (wet and dry) atmospheric inputs averaged below 100 mg TP m^{-2} y^{-1} (McCormick et al., 1999). The Everglades also received inflow waters from Lake Okeechobee occasionally, in minor amounts. Sawgrass (*Cladium jamaicense* Crantz) marsh, wet prairies, sloughs, and tree islands were among the vegetation communities present, and 65 to 70% of the total vegetation cover was under sawgrass (Loveless, 1959; Davis, 1989).

Approximately half of the 2 million hectares of the historic Everglades had been lost to agricultural as well as urban and suburban developments. The Everglades Agricultural Area (EAA), which occupies a large portion of the Florida Everglades, also was drained for agricultural purposes with soil losses occurring at an average rate of 3 cm yr^{-1} (Stephens, 1969). Unlike C and N, P released from the oxidation of these highly rich organic soils was either retained through sorption and precipitation or transported with the drainage water. A system of canals and pumps, which now encompass the water conservation areas (WCAs) surrounding the EAA, was constructed in the early 1960s for water supply, flood control, wildlife habitat, and recreational purposes (Fig. 1.2). Changes in hydrology and nutrient loading have significantly impacted large areas of the northern Everglades that shifted in vegetative cover from sawgrass to cattail communities. Overall productivity of the ecosystem was changed and, consequently, the long-term nutrient capacity of the marsh (Davis, 1991; Reddy et al., 1993; Craft and Richardson, 1993). Organic P accounts for at least 74% of soil P in the south Florida organic soils (Reddy, 1983; Koch and Reddy, 1992; Reddy et al., 1998). Fractionation procedures revealed that P is stored mainly as organic P (Qualls and Richardson, 1995; Reddy et al., 1998). Export of P from anthropogenic sources had led to increases in P availability in surface waters and acceleration of the internal recycling of previously stored P (Koch and Reddy, 1992).

Water Conservation Area 2A (WCA-2A) is a 447-km^2 region of the Everglades that exemplifies a wetland where P enrichment from external sources affects the

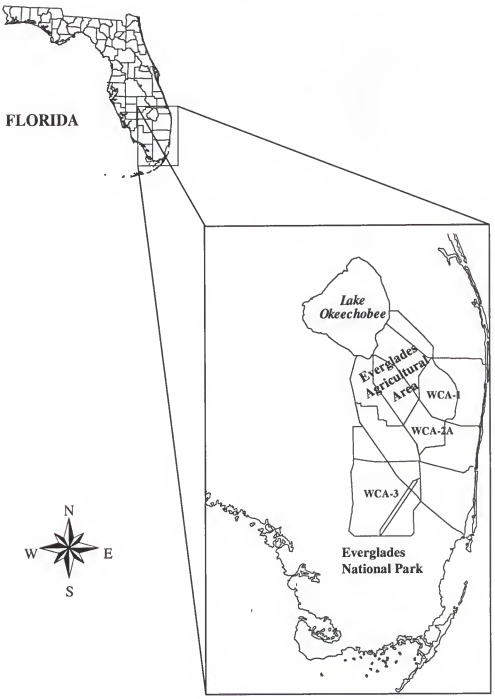

Fig. 1.2. A map of the Florida Everglades showing water conservation area-2A (WCA-2A) and other water conservation areas.

physicochemical and biological processes of the ecosystem. It is bounded on the northeast side by the Hillsboro Canal and on the southwest side by the North New River Canal. Both canals receive effluent water from the EAA. About 26% of the total area of WCA-2A had functioned as a P sink for the past three decades (Craft and Richardson, 1993). In this wetland organic P is about 70-90% of soil total P, with uptake by macrophytes and algae as the primary mechanism of P removal (Qualls and Richardson, 1995). In recent years, attention has been given to the exchange of P between sediment and the overlying water with respect to P_o (Newman and Reddy, 1993). To date, there is scant information on the mineralization of labile P_o from detrital and soil layers of a P-impacted wetland.

Objectives and Hypotheses

The main objective of this research was to determine the short-term mineralization of organic P in a freshwater wetland that has been impacted with P. This research should fill some of the information gaps in determining overall P cycling of the system and, ultimately, enable wetland managers to make sound decisions concerning the best water quality standards achievable for wetlands without compromising function of the wetland. The following specific questions will be addressed by the research:

- How does external loading of P affect the rate of labile P_o mineralization?
- How do extracellular enzymes and microbial activities affect the rate of P_o mineralization?
- How does the nature of P_o substrate regulate the mineralization of P_o in wetlands?

- How is the rate of P_o mineralization affected by the type of vegetation that contributes to the organic matter pool of wetlands?

Objective 1: Determine the effect of P loading and soil parameters on the mineralization of labile organic P.

Hypothesis: Phosphorus loading increases soil and porewater available P_i, which serves as a nutrient source for the microorganisms involved in degrading labile P_o in wetlands. Microbial biomass P, phosphatase activities, and substrate quality are among the soil parameters affecting P_o mineralization.

Objective 2: Determine the effect of inorganic electron acceptors on the rate of labile P_o mineralization in wetland.

Hypothesis: The rate of labile P_o mineralization is higher under aerobic than anaerobic conditions. The rate of labile P_o mineralization, however, is unaffected by the type of alternate electron acceptor (i.e., NO_3^-, SO_4^{-2}, or CO_2) unless an alternate electron acceptor is present at high concentrations in a wetland.

Objective 3: Determine the influence of substrate quality and extracellular enzyme (alkaline phosphatase) on the rate of labile P_o mineralization

Hypothesis: Alkaline phosphatase activity is higher in the non-impacted than P-impacted area, because significant amounts of available P_i present in the P-impacted area are repressible to enzyme activity, and low P_i availability in the unimpacted area can initiate biochemical mineralization of P_o.

Objective 4: Determine the rates of mineralization of native P_o and added labile P_o as affected by source of plant residues (i.e., cattail and sawgrass).

Hypothesis: The higher P present in cattail compared to sawgrass residues contributes to increases in mineralization of native and added labile P_o into soil incorporated with plant residues.

Dissertation Format

Each chapter of this dissertation was prepared as an individual manuscript intended for future publication. In this chapter, an overall introduction of wetland features and functions, nature of soil organic P, microbial and soil organic matter pools as contributors to soil P_o, and extracellular enzyme function with respect to P_o mineralization were presented. In Chapter 2, the influence of selected soil factors on the rate of labile P_o mineralization was investigated. In Chapter 3, the effect of inorganic electron acceptors (oxygen, nitrate, sulfate, and bicarbonate) on the mineralization rates of native and labile P_o was investigated. In Chapter 4, the mineralization of various P_o substrates in surface soils over time and the mineralization of P_o as affected by the phosphomono- and phosphodiesterase activities were examined in more detail. In addition, the influence of type of plant residues on P_o mineralization was determined. Finally, a summary of findings obtained and an integration of conclusions drawn from individual studies are included in Chapter 5 of the dissertation.

CHAPTER 2

INFLUENCE OF SOIL FACTORS ON ORGANIC PHOSPHORUS MINERALIZATION ALONG A EUTROPHIC GRADIENT IN THE FLORIDA EVERGLADES

Introduction

Organic P (P_o) is a major soil P pool in wetlands. The decomposition of organic matter can supply P to biotic organisms and, at the same time, influence the water quality of wetlands (Gale et al., 1992; Reddy and D'Angelo, 1994). Mineralization of P_o can occur through biological processes that are influenced by the rates of microbial growth and respiration or biochemical processes that involve the hydrolytic action of extracellular enzymes (McGill and Cole, 1981). Microorganisms are sources of extracellular and periplasmic enzymes or modifiers of soil conditions that influence the binding of P to organic matter (Wetzel, 1999). The soil microbial biomass is a small portion of soil organic matter but it has a short turnover rate and, therefore, can immediately supply P to the system (Jenkinson and Ladd, 1981).

The release of P bound to organic matter is also governed by the chemical composition of the substrate. The critical C:P ratio, defined as the ratio below which net mineralization occurs and above which net immobilization takes place, varied across several studies (Enwezor , 1976; Stevenson, 1986; Chesire and Chapman, 1996; Saggar et al., 1998). This is because underlying factors such as sedimentation rate, redox status,

moisture content, and nature of substrate could influence the C:P ratio of a substrate (Ingall and Van Cappellen, 1990; Amador and Jones, 1997; Bridgham et al., 1998).

Soil redox status can also affect P_o mineralization. Under anaerobic conditions, reduction of ferric iron associated with ferric phosphate results in the release of P_i (Patrick and Khalid, 1974). High amounts of P_i released as a result of soil anaerobiosis, however, can inhibit the activities of phosphatases and consequently reduce the hydrolysis of P_o (Dick and Tabatabai, 1978). Moreover, reduced species (e.g., Fe and Mn) that are by-products of soil reduction can also inhibit phosphatase activity (Juma and Tabatabai, 1977). Previous findings with respect to the influence of redox conditions on P_o mineralization are conflicting. Several studies indicated that more P_o is mineralized under aerobic than anaerobic conditions (Dick and Tabatabai, 1978; Brannon and Sommers, 1985; McLatchey and Reddy, 1998), while others obtained higher P mineralization under anaerobic than aerobic conditions (Campbell and Racz, 1975; Islam and Mandal, 1977; Ingall and Jahnke, 1994; Suzumura and Kamatani, 1995; Bridgham et al., 1998).

Phosphorus loading to an oligotrophic wetland can alter macrophyte community structure and cause changes in P storage pools of soil. For example, P loading to the Everglades Water Conservation Areas (WCAs) increased P accretion rates from 0.25 to 1.14 g P m^{-2} y^{-1} (Reddy et al., 1993). Changes in productivity that resulted from an increase in nutrient loading have brought changes in P storage for the organic pool (Qualls and Richardson, 1995; Reddy et al., 1998). It is probable that the mineralization of easily degradable forms of P_o may contribute to increased levels of inorganic P in certain areas of the WCAs.

Several physico-chemical and biological parameters are known to regulate P_o mineralization. The balance between P_o mineralization and the assimilation of inorganic P into microbial biomass (immobilization) depends on the quality of substrate, availability of O_2 or redox status, activities of extracellular enzymes and microbial biomass. It is, therefore, important to determine how these factors influence the mineralization of labile P_o in a wetland affected by an increase in nutrient loading, and also how these factors interact in regulating P_o mineralization.

The objectives of the study were to determine: (i) the influence of soil factor(s) that regulate(s) the hydrolysis of labile P_o in a wetland, and (ii) the relationship between P_o mineralization and soil factor.

Materials and Methods

Study Area

The historical Florida Everglades is a 700 km^2 oligotrophic freshwater wetland that received nutrients mainly from rainfall and periodic overflow from Lake Okeechobee (Davis, 1994). Over the last three decades, certain areas of the Everglades have been adversely affected due to changes in hydrology and an increase in nutrient loading. This impact caused a shift in vegetation communities from sawgrass (*Cladium jamaicense* Crantz) and open water slough to cattail (*Typha* spp.) (Davis, 1991; Rutchey and Vilchek, 1994) as well as soil P enrichment (Reddy et al., 1993; De Busk et al., 1994; Newman et al., 1997; Reddy et al., 1998).

Water Conservation Area 2A (WCA-2A) is a 447 km^2 impounded marsh in the northern Everglades. The Hillsboro Canal encompasses the marsh on the northeast while the North New River Canal surrounds it on the southwest (Fig. 2.1). Surface water flows to WCA-2A through four S-10 control structures located along the Hillsboro Canal, and through pump station S-7 on the North New River Canal. About 58% of the water entering WCA-2A originated from the EAA (South Florida Water Management District, 1992). There is a distinct nutrient gradient that traverses approximately 10 km, from north of inflow structure S-10C to the south (the interior of the marsh). Eight sampling stations at 1.4, 2.3, 3.2, 4.2, 5.1, 7, 8.4, and 10.1 km from the inflow structure were established along this north to south nutrient gradient (Fig. 2.1).

Soil Sampling

Soil cores were collected at all eight stations for three sampling intervals - February 1995 (dry season), August 1996 (wet season), and March 1997 (dry season). Water depths varied seasonally from <2 cm to >2 m. Detritus covering the surface soil was removed, cut into 2-5 cm lengths, and mixed thoroughly to obtain a representative sample. After the surface cover has been removed, five cores were collected at each station using a thin-walled 10-cm diameter aluminum coring tube. Only cores which had < 5% compaction as previously determined by a probe were collected. Cores were capped, removed from the ground, immediately extruded, and separated into 0-10 and 10-30 cm depths. All cores from each station were composited by depth intervals, live roots were removed, and dead roots were cut into small sections before mixing. Each composite detritus/soil sample was used for field incubation and subsequent laboratory studies.

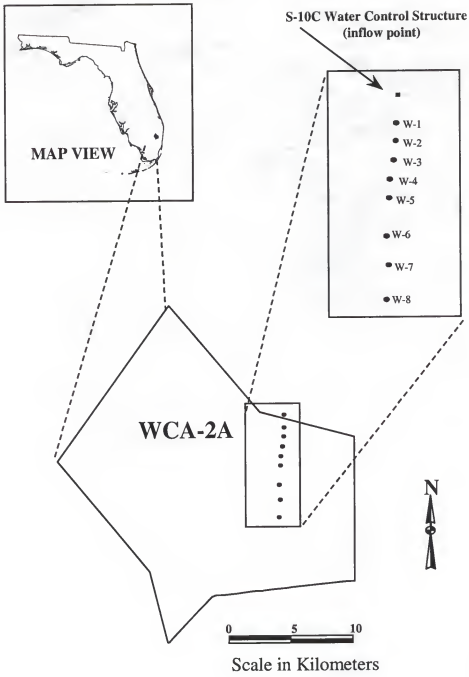

Fig. 2.1. Map showing sampling locations along a eutrophic gradient in Water Conservation Area-2A of the Everglades.

Samples were divided into three portions. A portion was spread onto a 25-cm × 20-cm Type A/E Gelman glass filter, and allowed to drain for 15 min to remove water, and was designated as "drained". The second portion was maintained under saturated soil conditions, and was designated as "flooded". The remaining portion of sample was sealed in plastic bags and kept on ice until transported to the laboratory for storage at 4°C. The drained and flooded samples were analyzed for *in situ* substrate-induced P mineralization (SIPM).

To determine variability, five individual soil cores were obtained from each of the stations located at 2.3, 7, and 10.1 km from inflow. The cores were taken about 10 m apart from each other. Detritus and the surface 0-10 cm soil were subdivided and analyzed for total P content, microbial biomass P, SIPM, and potentially mineralizable-P (PMP).

Organic P Fractionation

Phosphorus was separated into microbial biomass and various organic pools using the procedure of Ivanoff et al. (1998) with some modifications as follows:

Microbial Biomass P (MBP) and Labile P_o:

Half-gram dry equivalent of sample was weighed into a 50-mL centrifuge tube in duplicates. First tube was immediately equilibrated with 25 mL of 0.5 M $NaHCO_3$ (pH=8.5) for a shaking period of 16 h. Suspension was centrifuged at 4066 × *g* for 10 min and supernatant was passed through a 0.45 µm membrane filter. Filter that had a 100-fold smaller pore-size than the suggested Whatman no. 41 filter paper was used to exclusively obtain labile P_o, which are characteristically of lower molecular weights.

The second tube was added with 1 mL of ethanol-free $CHCl_3$ and capped for 24 h with intermittent mixing. Tube was uncapped in a fume hood for 24 h. When $CHCl_3$ has been driven off, sample was equilibrated with 0.5 M $NaHCO_3$ and filtered as described previously. The residue of the $CHCl_3$-treated sample was saved for further fractionation.

An aliquot of the filtrate was analyzed for total P after $K_2S_2O_8$ oxidation followed by H_2SO_4 digestion (Bowman, 1989). Filtrate of the control sample was analyzed for soluble reactive P (SRP) by the Murphy and Riley (1962) method. Microbial biomass P was calculated as the difference in total P between the $CHCl_3$-treated and control $NaHCO_3$ extracts (Hedley and Stewart, 1982). Values were reported without correction for efficiency. Total labile P_o content, on the one hand, was calculated as the difference between the TP of the $CHCl_3$-treated and SRP of the control $NaHCO_3$ extracts.

Inorganic P:

Residue from the previous extraction was weighed and added with 25 mL of 1 M HCl. Suspension was equilibrated for 3 h with continuous shaking. Tubes were centrifuged and filtered as described previously. Filtrate was analyzed for SRP using an autoanalyzer (USEPA, 1993 Method 365.4). Remaining HCl was rinsed off from residue by shaking with distilled water for 30 sec instead of 5 min suggested in Ivanoff et al. (1998). Suspension was centrifuged and filtered as described previously. Water extracts were analyzed for SRP and result was added to the 1 M HCl extractable-P_i pool.

Moderately Resistant Organic P:

Residue from previous extraction was weighed before added with 25 mL of 0.5 M NaOH. Suspension was equilibrated for 16 h with an end-to-end shaking. When equilibrated, tube was centrifuged and supernatant filtered as described previously. A

portion of the extract was acidified to pH<2.0 with ultrapure concentrated H_2SO_4 and the clear liquid was analyzed for SRP and TP. An unacidified portion of the extract was analyzed for TP after $K_2S_2O_8$ oxidation followed by H_2SO_4 digestion (Bowman, 1989).

Highly Resistant Organic P:

Residue in centrifuge tube was transferred in a previously weighed 50-mL beaker and was dried at 70°C to a constant weight. The dried sample was ashed at 550°C for 4 h and the ash dissolved in 6 M HCl (Andersen, 1976).

Potentially mineralizable-P (PMP)

A 0.5-g dry equivalent of fresh detritus or soil was weighed into 50-mL polyethylene tubes having caps equipped with rubber septa in triplicates. Two mL distilled water was added to each tube to create flooded conditions. The capped tube was evacuated for 10 sec and purged with O_2-free N_2 gas for 3-4 min. The tubes were incubated at 40°C for 10 d. After incubation, each sample was equilibrated with 25 mL 0.5 M $NaHCO_3$ (pH = 8.5) by end-to-end shaking for 30 min. When equilibrated, tube was centrifuged at 4066 × *g* for 10 min, and the supernatant was passed through a 0.45 µm membrane filter (Gelman Sciences, Ann Arbor, MI). The filtrate was analyzed for SRP by the Murphy-Riley (1962) method using an ultraviolet-visible spectrophotometer (UV-160, Shimadzu Corp., Japan) set at 880 nm.

Control samples were weighed into 50-mL polyethylene tubes in triplicates. Two mL of distilled water was added to each tube before sample was equilibrated with 0.5 M $NaHCO_3$ and filtered in the same manner as described previously.

Potentially mineralizable-P excluding any precipitation of mineralized P from P_o was calculated as follows:

$$PMP = [(NaHCO_3\text{-}P_i)_{t=10} - (NaHCO_3\text{-}P_i)_{t=0}]/t$$

where $(NaHCO_3\text{-}P_i)_{t=10}$ is the bicarbonate-extractable inorganic P after the 10-d incubation; $(NaHCO_3\text{-}P_i)_{t=0}$ is the bicarbonate-extractable inorganic P at the onset of incubation; t is the length of incubation period in d; and PMP is the potentially mineralizable-P in mg kg^{-1} d^{-1}.

The soil that remained after the 0.5 M $NaHCO_3$ extraction was subsequently equilibrated with 1 M HCl for 3 h to account for the precipitation of P mineralized from P_o. Potentially mineralizable-P was calculated as follows:

$$PMP = \{[(NaHCO_3\text{-}P_i)_{t=10} + (HCl\text{-}P_i)_{t=10}] - [(NaHCO_3\text{-}P_i)_{t=0} + (HCl\text{-}P_i)_{t=0}]\}/t$$

where $(NaHCO_3\text{-}P_i)_{t=10}$ and $(HCl\text{-}P_i)_{t=10}$ are the bicarbonate-extractable inorganic P and the acid-extractable inorganic P, respectively, after the 10-d incubation; $(NaHCO_3\text{-}P_i)_{t=0}$ and $(HCl\text{-}P_i)_{t=0}$ are the bicarbonate-extractable inorganic P and the acid-extractable inorganic P, respectively, at the onset of incubation; t is the length of incubation period in days; and PMP is the potential mineralizable-P, mg kg^{-1} d^{-1}.

Substrate-induced P mineralization (SIPM)

Substrate-induced P mineralization, a technique similar to substrate-induced respiration, is a measure of ambient microbial/enzyme activity (Anderson and Domsch, 1978a; West and Sparling, 1986). Using glucose-6-phosphate, SIPM was determined for detritus and soils under natural "flooded" or artificial "drained" conditions. About 5 g of the flooded sample was weighed into six pre-weighed 50-mL polyethylene centrifuge tubes and approximately 5 g of the drained sample was weighed in six pre-weighed 250-

mL wide-mouth polyethylene bottles. Each set of six was divided into three controls and three P_o-treated samples. To each treated sample, 500 μL of 200 mg P L^{-1} disodium salt of *D*-glucose-6-phosphate (Sigma Chemical Co., St. Louis, MO) solution was added. The flooded sample was purged with N_2 for 5 min to induce anaerobic conditions before incubation. The samples were incubated with site water in coolers at ambient temperature (29°C) for 4 h. After incubation, samples were weighed and equilibrated with 25 mL of 0.5 M $NaHCO_3$ solution (pH = 8.5) for 30 min under continuous end-to-end shaking. The suspensions were filtered through 0.45-μm membrane filters. The filtered solutions were stored at 4°C before analysis. Soluble reactive P was determined in filtrates by the Murphy and Riley (1962) method using an ultraviolet-visible spectrometer (UV-160, Shimadzu Corporation, Japan). The difference in SRP between the control and the glucose-6-P-treated samples after the 4-h incubation represents SIPM. This difference was normalized to the total amount of P_o added, as follows:

$$SIPM = [(NaHCO_3\text{-}P_i)_{t=4} - (NaHCO_3\text{-}P_i)_{t=0}] / [t \times (S\text{-}P_o)_{t=0}]$$

where $(NaHCO_3\text{-}P_i)_{t=4}$ is the bicarbonate-extractable inorganic P after the 4-h incubation, mg kg^{-1}; $(NaHCO_3\text{-}P_i)_{t=0}$ is the bicarbonate-extractable inorganic P at the beginning of incubation, mg kg^{-1}; $(S\text{-}P_o)$ is the glucose-6-P added, mg kg^{-1}; t is the incubation time, h; and SIPM is the substrate-induced P mineralization rate, $hour^{-1}$.

The SIPM rates were also determined following laboratory incubation of samples collected in August 1996 under both natural "flooded" and artificial "drained" conditions. Samples that were previously stored at 4°C were pre-incubated at room temperature for 24 h before SIPM analysis. Drained samples were prepared and incubated as described under field incubation. After incubation, flooded or drained samples were equilibrated

with 25 mL of 0.5 M $NaHCO_3$ for 30 min under continuous end-to-end shaking. Samples were centrifuged at 4066 × *g* for 10 min, and the supernatant was filtered through a 0.45-μm Gelman membrane filter. Filtered samples were stored at 4°C until analysis. Soluble reactive P was determined in the same manner as the field-incubated samples. The SIPM rates were calculated as described previously.

Total C, N, P, and Ash Content

Finely ground dried subsamples were analyzed for total C and N using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). A 0.5-g dried subsample was ashed at 550°C for 4 h and ash dissolved in 6 M HCl to determine total P (TP) (Andersen, 1976). The digestate was analyzed for SRP using an autoanalyzer (USEPA, 1993 Method 365.4). Alternately, TP was analyzed using HNO_3-$HClO_4$ digestion (Sommers and Nelson, 1972). This method showed a good correlation ($r^2 = 0.97$) with the ashing method in the August 1996 samples.

Statistical Analyses

The SIPM and PMP rates were correlated with total P, total labile P_o, $NaHCO_3$-P_i, alkaline phosphatase activity (APA), mass C:P_o ratio, and microbial biomass P by depth and season using regression analyses in Microsoft Excel Version 97. Observations for all depths were combined when two out of three depths in each season showed a similar trend, and regression analyses were conducted. Data were best fitted to linear, logarithmic or exponential equations.

Results and Discussion

Detritus/Soil Characterization

Bulk density in the 0-10 cm soil ranged from 0.052-0.097 g cm^{-3} and averaged 0.069 g cm^{-3}, with 20% coefficient of variation along the transect (Table 2.1). Bulk density in the 10-30 cm soil was generally higher than for the 0-10 cm soil, and decreased with distance from inflow. Ash content of the detritus obtained at 7 to 10 km was higher than at distances near the inflow. Detritus at the interior of the marsh consisted of dead sawgrass plants attached with calcareous periphyton (Browder et al., 1994). The periphyton mats effectively bind phosphates through co-precipitation with $CaCO_3$ (Otsuki and Wetzel, 1972). There was no trend, however, observed with respect to ash content with distance for the 0-10 and 10-30 cm soils.

Total C contents averaged across all sampling stations were 429±5, 436±6, and 460±5 g kg^{-1} for the detritus, 0-10 cm and 10-30 cm soils, respectively. The mass C:P ratio, however, varied widely from 259:1 to 2096:1. This implies P limitation in the system. Average mass C:P ratios for the 10-30 soil (793:1 to 2096:1) were above the critical value of 300:1 (Stevenson, 1986), therefore indicating net P immobilization at this soil depth. In contrast, mass C:P ratios obtained for detritus from near the water inflow extending up to 4 km, and in 0-10 cm soil sampled at station less than 2 km from the inflow were below the critical level. Total N contents averaged across all stations were 23±5, 29±1, 30±1 for the detritus, 0-10 cm, and 10-30 cm soils, respectively.

Microbial Biomass P

Soil microbial biomass is a relatively labile component of soil organic matter and susceptible to mineralization through $CHCl_3$ fumigation (Jenkinson and Ladd, 1981;

Brookes et al., 1982; Hedley and Stewart; 1982; Brookes et al., 1984; Sparling et al., 1985). Subsequent removal of P from lysed microbial cells using extractants such as 0.5 M $NaHCO_3$ can give a measure of P held in the biomass.

Microbial biomass P was 20-37, 7-16, and 7-14% of total P for the detritus, 0-10 cm soil, and 10-30 cm soil, respectively. The coefficient of variation in microbial P for the average of three stations was 19% for the detritus and 34% for the 0-10 cm soil (Table 2.2). McLatchey and Reddy (1998) obtained comparable microbial biomass P of 6-18% based on TP, with coefficients of variation ranging from 17-28% in surface soils of a restored wetland. Likewise, Ivanoff et al. (1998) obtained 6-21% of TP as microbial biomass P in 0-10 cm soils of selected Histosols from the Everglades Agricultural Area (EAA). Thus, microbial biomass is a significant pool of soil P in wetlands.

Microbial P generally decreased with soil depth, and was negatively correlated with distance from inflow for the detritus and 10-30 cm soil ($P<0.05$). Highly significant ($P<0.01$) negative correlation of microbial P with distance, however, was obtained for the 0-10 cm soil. Qualls and Richardson (1995) observed no correlation of microbial P in the 0-5 cm soil increment with average distance along three transects in WCA-2A. These researchers reported microbial P values that had been corrected for an analytical efficiency of only 43%. Spatial dependence of the correction factor probably obscured any relationship sought with distance for microbial biomass P. However, the corrected microbial P values they obtained for the 0-5 cm soils, ranging from 17 to 37% of TP, are comparable to the range of values (17-39%) we obtained for the 0-10 cm soil if corrected for 40% efficiency.

While most researchers correct their values for efficiency that they designate as k_p (Hedley and Stewart, 1982; Brookes et al., 1982; Walbridge, 1991), we chose to interpret the data uncorrected. Efficiency-corrected microbial P values, when expressed as a percent of total P, showed as much as 98% of TP in this pool for the detritus collected 7 km from the inflow (Fig. 2.2*a*). This is probably due to lysing of detrital tissue, resulting in an overestimation of microbial biomass P. McLaughlin and Alston (1985) showed that during a 16-h extraction, fresh root or shoot material added to soils before $CHCl_3$ fumigation overestimates measurement of microbial biomass P. High labile organic matter in surface soils of the WCAs can also overestimate microbial biomass P (Reddy et al., 1998). Most studies determined conversion factor by adding selected bacterial and fungal cultures to soils. Using suspended bacterial and fungal cells labelled with ^{32}P, Eberhardt et al. (1996) observed wide variations (0.44 to 0.84) in conversion factors among organisms. The type and amount of each type of microorganism, therefore, affect the extractable part of the microbial biomass P. Beare et al. (1990) observed that fungi tend to dominate over bacteria during early stages of plant residue decay, and that the ratio of fungal to bacterial biomass can vary from 1:1 to 11:1 (McLaughlin et. al., 1986). Hedley and Stewart (1982) suggested a k_E value of 0.4 for soils with pH ranging from 6.2 to 8.2. While our samples had circumneutral pH, the k_p derived from calibrations with mineral soils using two bacteria and two fungal cultures may not be applicable to the detritus and organic soils of WCA-2A. Therefore, there is need to develop an efficiency-factor calibration in order to compare values obtained across several ecosystems.

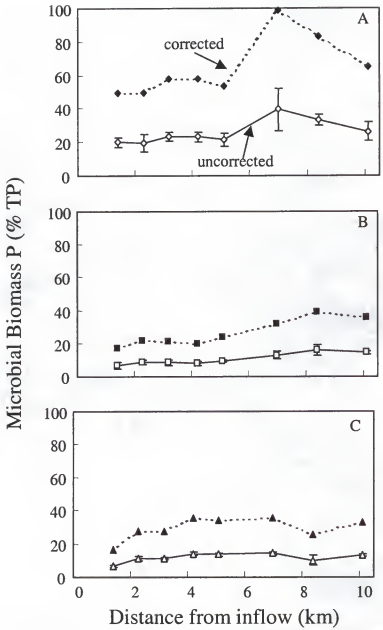

Fig. 2.2. Microbial biomass P as a function of distance for (a) detritus, (b) 0-10 cm soil, and (c) 10-30 cm soil. Unshaded and shaded symbols denote uncorrected values and values corrected for an efficiency of 0.40 (Hedley and Stewart, 1982).

Table 2.1. Physico-chemical and microbiological characteristics of detritus and soils along a 10-km transect from north to south of S10-C in WCA-2A.

Distance	Bulk Density[†]	Ash content	Microbial P[‡]	Total P
km	g cm^{-3}	%	----------mg kg^{-1}----------	
Detritus				
1.4	nd[§]	9.2 ±1.9	314 ±48	1601 ±165
2.3	nd	9.8 ±1.9	323 ±89	1615 ±102
3.2	nd	9.4 ±0.8	348 ±35	1509 ±70
4.2	nd	10.0 ±2.2	337 ±67	1456 ±265
5.1	nd	8.9 ±0.8	232 ±41	1106 ±153
7.0	nd	13.8 ±6.0	320 ±87	859 ±145
8.4	nd	12.1 ±2.4	190 ±49	556 ±102
10.1	nd	17.9 ±1.2	110 ±27	417 ±14
0-10 cm				
1.4	0.056	18.4 ±4.0	108 ±34	1552 ±59
2.3	0.066	11.1 ±0.6	119 ±18	1369 ±58
3.2	0.064	11.6 ±0.1	102 ±21	1205 ±61
4.2	0.097	12.2 ±0.3	80 ±15	989 ±83
5.1	0.078	12.5 ±0.4	92 ±7	966 ±35
7.0	0.069	17.9 ±4.0	85 ±23	641 ±88
8.4	0.067	11.5 ±0.7	80 ±28	489 ±64
10.1	0.052	12.9 ±0.6	71 ±17	479 ±71
10-30 cm				
1.4	0.104	13.2 ±0.3	38 ±7	570 ±57
2.3	0.116	10.6 ±0.1	41 ±5	373 ±6
3.2	0.099	10.3 ±0.9	40 ±5	363 ±39
4.2	0.095	11.0 ±0.3	40 ±2	287 ±22
5.1	0.076	13.2 ±1.8	40 ±5	289 ±18
7.0	0.098	21.4 ±2.9	34 ±5	247 ±48
8.4	0.088	9.5 ±0.2	23 ±7	228 ±8
10.1	0.065	9.4 ±0.3	32 ±3	246 ±18

[†] weight of dry soil in 10-cm diameter aluminum core

[‡] difference between total P of 0.5 M $NaHCO_3$ extracts of $CHCl_3$-treated and non-treated samples; values uncorrected for extraction efficiency.

[§] not determined.

All values except for bulk density are an average for three sampling events ± standard error.

Table 2.2. Variability among replicate soil cores obtained from selected locations in WCA-2A. (n = 5 soil cores/station; MBP = microbial biomass P; PMP = potentially mineralizable-P; SIPM = substrate-induced P mineralization)

Parameter	Distance from inflow	Detritus	0-10 cm soil
	km	Coefficient of variation (%)	
MBP[†]	2.3	25	44
	7.0	22	23
	10.1	9	36
		19±5[‡]	34±6
Total P	2.3	11	24
	7.0	14	14
	10.1	15	9
		13±1	16±5
PMP[§]	2.3	27	80
	7.0	15	35
	10.1	20	49
		21±3	55±13
SIPM[§]	2.3	85	137
	7.0	43	46
	10.1	117	15
		82±21	66±37

[†] Values uncorrected for extraction efficiency

[‡] Means of three stations ± standard error

[§] Determined from net change in $NaHCO_3$-extractable P_i only

Total P and Labile Organic P

Total P decreased with soil depth and distance from the inflow ($P < 0.01$) (Table 2.1). Soil TP decreased exponentially with distance for the 0-10 and 10-30 cm soils (Fig. 2.3). Total P at 1.4 km from the inflow was three to four times higher than at a station approximately 10 km from the inflow for the detritus and the 0-10 cm soil, while it was two times higher between the two stations for the 10-30 cm soil. The coefficient of variation in total P averaged for three stations was 13% for the detritus and 16% for the 0-10 cm soil (Table 2.2).

Labile P_o is defined as the P_o pool extracted by mild reagents such as 0.5 M $NaHCO_3$ and assumed as a rapidly mineralized P pool (Bowman and Cole, 1978a). Total labile P_o calculated as the difference between the TP of the fumigated extract and SRP of the non-fumigated extract, and decreased with soil depth. Total labile P_o averaged 29% of TP (20-41%) in the detritus, 13% (8-22%) in the 0-10 cm soils, and 16% (8-20%) in the 10-30 cm soil during the dry season (Table 2.3). During the wet season, this P pool was 23% (14-34%), 12% (8-18%), and 14% (7-16%) of TP for the detritus, 0-10 cm, and 10-30 cm soils, respectively (data not shown). Total labile P_o did not show a significant correlation with distance for any depth during the dry season; however, there was a negative correlation ($r = -0.72$-0.79; $P < 0.05$) with distance for all three depths during the wet season. Increase in moisture content during the wet season probably enhanced the release of labile P_o, and the decrease of this P pool with distance indicated differences in substrate quality (i.e., between cattail and sawgrass). Labile P_o compounds are produced under conditions favorable for both microbial and biochemical degradation that include high moisture content and temperature (Sumann et al., 1998).

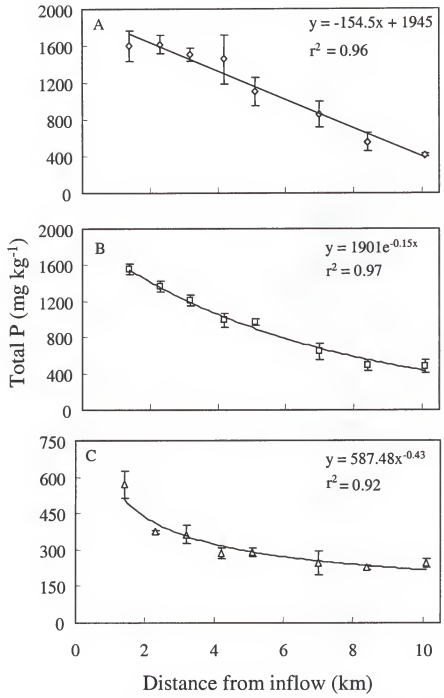

Fig. 2.3. Total P as a function of distance for (a) detritus, (b) 0-10 cm soil, and (c) 10-30 cm soil.

Total P showed a positive correlation ($r = 0.80$; $P<0.05$) with total labile P_o in the detritus only during the dry season and in both the detritus ($r = 0.75$; $P\leq0.05$) and the 0-10 cm soil ($r = 0.81$; $P<0.05$) during the wet season. Detritus that accumulates at the surface is an important source of labile P_o (Reddy et al., 1993) and the changes in productivity may have contributed to differences in the labile P_o pool together with TP. At the 0-10 cm depth, however, the high water table increased moisture content resulting in a greater release of labile P_o during the wet rather than dry season.

The difference between total labile P_o and microbial biomass represents the labile P_o associated with dead organic forms of plant roots and of microbial origin (Reddy et al., 1998). For the detritus, it was 2-26% of total labile P_o and negatively correlated ($P<0.01$) with distance from the inflow. This difference was 8-37% and 12-22% of total labile P_o for the 0-10 cm soil and 10-30 cm soils, respectively. However, labile P_o associated with dead organic was negatively correlated ($P<0.05$) with distance at the 0-10 cm depth but not at the 10-30 cm depth. Thus, there is a significant portion of P associated with dead organic forms in WCA-2A that can contribute to the P_i pool after mineralization.

Labile and Moderately Labile Inorganic P

Labile P_i that is extracted by 0.5 M $NaHCO_3$ is loosely adsorbed and immediately available. An increase in solubility of calcium phosphates as a result of decreases in calcium ion activity in solution formed the basis for this extraction procedure (Bowman and Cole, 1978b). This fraction decreased with depth and was less than 1 to 3% of TP (Table 2.3). Relatively high labile P_i content was found for the detritus up to 5 km from the inflow. The high amounts of labile P_i in the detritus and 0-10 cm soils, particularly at stations near the inflow, suggest that inorganic P may be loosely bound to organic matter

or present as recently precipitated amorphous, monocalcium phosphate (Reddy et al., 1998). The HCl-P_i fraction represents Ca- and Mg-bound P and is considered to be moderately labile (Ivanoff et. al., 1998). This fraction did not show significant correlation with distance. However, there were higher amounts of HCl-P_i in detritus and 0-10 soil compared to the 10-30 cm soil. This suggests precipitation of surface SRP as calcium phosphate because of the presence of high amounts of calcium in drainage water entering WCA-2A. Such precipitation is enhanced when floodwater pH is above 8 (Diaz et al., 1994; Reddy et al., 1998).

Moderately Resistant Organic P

The total P extracted by 0.5 M NaOH is composed of fulvic and humic acid P. Fulvic acid P_o is considered moderately labile and, together with fulvic acid P_i, is separated from humic acid P after acidification to pH < 2. Assuming 13% of soil TP in the P-enriched areas (up to 5 km from inflow) to be NaOH-P_i (Reddy et al., 1998), about 55% of total NaOH-P was NaOH-P_o in these areas. Sodium hydroxide extractable P_o has a slow turnover and, therefore, considered as moderately stable. It can be a source for microbial P uptake when P_i is limiting (Robinson et al., 1998).

Highly Resistant Organic P

Residual P_o is a highly resistant and non-bioavailable P pool, and residual P_o as percent of TP increased with soil depth. Residual organic P is 1-37% of TP in the detritus, 13-44% of TP in the 0-10 cm soil, and 40-56% of TP in the 10-30 cm soil. A high percentage of TP as residual P_o in the 10-30 cm soil suggests that most P in this depth is associated with stable organic materials such as lignin and organometallic complexes (Ivanoff et al., 1998). There was significant ($P<0.01$) negative correlation

Table 2.3. Phosphorus distribution among pools for samples of detritus and soils collected during the dry season (March 1997).

Distance	$NaHCO_3$-P_i	Total labile P_o	HCl-P_i	NaOH-TP	Residual P_o	Total P
km			mg kg^{-1}			
Detritus						
1.4	38±4	379	188±9	589±5	707	1901
2.3	48±2	453	167±8	462±0	675	1805
3.2	40±3	378	207±11	468±29	541	1633
4.2	68±5	492	281±20	550±25	568	1960
5.1	35±2	325	160±4	448±17	444	1412
7	3±1	459	135±20	241±6	272	1110
8.4	6±1	272	210±68	198±4	7	693
10.1	6±0	158	103±5	106±0	71	445
0-10						
1.4	22±3	124	370±16	440±14	625	1581
2.3	12±0	124	268±27	399±13	594	1397
3.2	13±1	147	218±12	370±25	577	1324
4.2	2±1	86	155±10	279±4	353	877
5.1	14±1	97	214±10	281±17	368	974
7	12±0	120	364±122	162±13	96	755
8.4	5±0	139	92±6	141±1	240	616
10.1	7±0	112	154±48	156±11	193	621
10-30						
1.4	11±1	44	70±2	148±8	276	549
2.3	10±0	54	33±8	115±5	157	368
3.2	7±0	56	22±1	117±6	218	419
4.2	4±1	52	9±1	78±3	136	279
5.1	3±1	63	14±0	85±3	153	317
7	2±1	44	26±1	41±3	117	231
8.4	5±0	46	24±9	67±3	94	237
10.1	3±1	50	10±1	57±2	152	273

Values ± standard error among triplicate analyses.

obtained for residual P_o with distance for both detritus and 0-10 cm soil, though none for the 10-30 cm soil. This suggests that there is more highly resistant P_o from cattail than sawgrass or peripyton commnunities.

Potentially mineralizable-P (PMP)

The PMP rate was higher in the detritus compared to the 0-10 or 10-30 cm soils. The coefficients of variation for PMP averaged from three stations were 21% for the detritus and 55% for the 0-10 cm soil (Table 2.2). Higher variability in the surface soil than the detritus can be explained by the soil being heterogeneous and even mixed with plant materials that are undergoing varied degrees of decomposition.

Potentially mineralizable-P rates calculated from the net change in $NaHCO_3$-P_i were lower than rates estimated from the net change in combined $NaHCO_3$- and HCl-extractable P_i (Fig. 2.4). The rate calculated from the change in the combined P_i pools was twice that of the rate calculated from $NaHCO_3$-P_i alone. Thus, it is likely that a significant portion of P_i released during P_o mineralization was precipitated as calcium phosphate, as soils from WCA-2A have high levels of extractable calcium (Reddy et al., 1993; DeBusk et al., 1994; Reddy et al., 1998). Therefore, PMP rates calculated from net changes in the combined $NaHCO_3$- and HCl-extractable P_i will be considered in the subsequent discussion.

The PMP was significantly correlated to microbial biomass P ($r^2 = 0.81$) (Fig. 2.5*a*), with the turnover of microbial biomass P being estimated at 13 d. Using ribonucleic acid (RNA) as a substrate, McLatchey and Reddy (1998) obtained turnover times of 17 h for a wetland organic soil incubated at 25°C under aerobic conditions, and between 1 to 4 d for soils incubated with alternate electron acceptors (NO_3^-, SO_4^{-2}, or

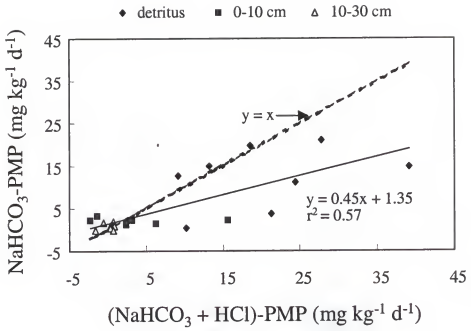

Fig. 2.4. Potential mineralizable-P (PMP) calculated as combined $NaHCO_3$ + HCl-P_i as a function of PMP expressed as $NaHCO_3$-P_i alone of samples in March 1997.

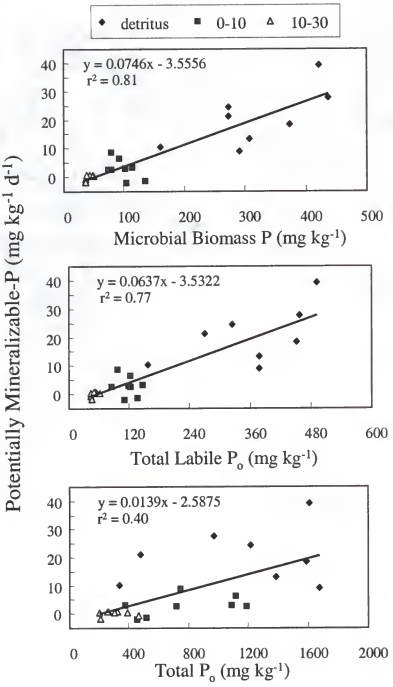

Fig. 2.5. Potentially mineralizable-P (PMP) calculated from both $NaHCO_3$- and HCl-P_i as a function of (a) microbial P, (b) total labile P_o, and (c) total P_o of samples in March 1997.

HCO_3^-). Although the incubation temperature used in this experiment was 15°C higher than that of McLatchey and Reddy, we obtained a longer turnover of microbial biomass P than by these latter researchers. Not all microbial biomass P is probably as labile as RNA. In addition, the soil examined by McLatchey and Reddy (1998) had a low pH and, therefore, is dominated by fungi that have a lower cell wall to cytoplasm ratio compared to bacteria. Cell walls are more resistant to degradation than cytoplasm (Anderson and Domsch, 1978b). Thus, the recycling of P from the microbial pool in WCA-2A wetland soils can take place over periods ranging from days to weeks (Qualls and Richardson, 1995).

Total labile P_o when plotted as a function of PMP showed a significant positive correlation ($P<0.01$, n=24) (Fig. 2.5*b*). The reciprocal of the slope for the line of 16 days represents the turnover time for this P_o pool. In comparison, Bridgham et al. (1998) obtained turnover times for labile P_o in northern wetlands in the range of 14 to 91 d under anaerobic and aerobic conditions. Penn et al. (1995) reported that the turnover times of labile P_o in the upper 1 cm of Onondaga Lake sediment was 4.8 per year while it was 0.11 per year for lake sediment located approximately 30 cm. Hence, labile P_o in upper sediments hydrolyzed faster than in underlying sediments.

Residual P_o was not correlated with PMP at all depths. It is probable that the incorporation of this P_o pool into humic substances caused it to be resistant to hydrolysis (Brannon and Sommers, 1985). In contrast, a plot of total P_o vs. PMP showed a significant positive relationship ($P<0.05$) with a slope of 0.0139 per day or a turnover rate of 72 d (Fig. 2.5*c*). Thus, in WCA-2A, it takes more than two months for the total P_o pool to be converted to soluble P_i.

The turnover times for the microbial P and total labile P_o pools obtained in this study indicated rapid recycling of substantial portions of total soil P. Hydrologic fluctuations in wetlands such as WCA-2A can partly contribute to the turnover of P pools by shifting the microbial population from aerobic to anaerobic and vice versa.

There was a positive correlation ($P \leq 0.01$; n=24) of PMP with $NaHCO_3$-P_i. Thus, the presence of more available P_i has provided more nutrient source for microorganisms to convert P_o into P_i. Fenchel and Harrison (1976) observed that P_i enrichment increases the number of bacteria as well as higher trophic organisms, thereby, increasing rates of decomposition. Thus, the $NaHCO_3$- and NaOH-extractable P_o represented potentially mineralizable-P_o pools.

When mass C:P_o ratios in all depths were plotted against PMP (Fig. 2.6), a significant correlation ($P \leq 0.01$) was obtained. Based on this plot, the critical C:P_o ratio for net mineralization is between 300 and 500 for detritus and soil of WCA-2A. Likewise, Saggar et al. (1998) reported a critical C:P ratio of 550 for forest soils. High C:P_o mass ratios (960 to 2194) in the 10-30 cm soils suggested net immobilization.

In addition to substrate quality, enzymes particularly phosphatases, can regulate P_o mineralization in wetlands. However, alkaline phosphatase activity (APA) values obtained from Wright and Reddy (2000), did not correlate with PMP. Similarly, Harrison (1982b) obtained no significant relationship between phosphatase activity and rate of ^{32}P-RNA mineralization for fifty woodland soils. Aside from soil properties, the activities of soil phosphatases are regulated by biogeochemical factors such as soil depth, season, vegetation, and parent material (Harrison, 1983). The involvement of several enzymes and linkage between the substrates these enzymes utilize can confound the relationship

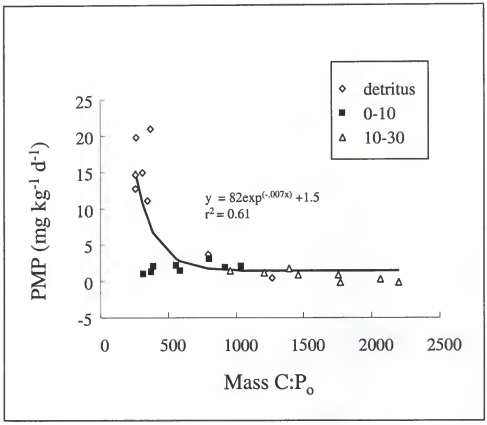

Fig. 2.6. Potential mineralizable-P as a function of mass $C:P_o$ ratio for detritus and soils collected on March 1997. n = 24

between phosphatase activity and P_o mineralization (Marsden and Gray, 1986). It is also probable that our findings were confounded by the effects of dessication resulting from the 10-d incubation at 40°C that reduced the activities of phosphatase. Air-drying has been reported to reduce the activity of this enzyme (Eivazi and Tabatabai, 1977; Speir and Ross, 1981). Inorganic P released after air-drying (Marumoto et al., 1982; Sparling et al., 1985; Qiu and McComb, 1995) can inhibit the activity of phosphatases. While Nannipieri et al. (1982) showed the resistance of acid phosphatases to thermal denaturation up to 80°C, this might not be true for alkaline phosphatases since every enzyme forms a distinct complex with the soil matrix. Kandeler (1990) observed that alkaline phosphatases are inactivated at 50°C, and adsorption of phosphatases to organic matter probably can result to greater thermal stability of the enzyme.

Substrate-induced P mineralization (SIPM)

The use of labile substrates such as glucose-6-P and short-term incubation represent P_o mineralization at ambient microbial and enzyme activities. This concept is similar to the substrate-induced respiration methods used by several researchers for evaluating ambient microbial activities (Anderson and Domsch, 1978a; West and Sparling, 1986).

The SIPM rates for wet-season drained samples measured under a constant temperature of 30°C in the laboratory plotted against in situ rates showed a significant ($r = 0.79$, $n = 24$) logarithmic relationship (Fig. 2.7). Rates measured under laboratory conditions were lower than those measured under field conditions, suggesting the influence of storage on enzyme activities (DeLuca et al., 1992; Coxson and Parkinson, 1987).

In a 4-h incubation, mineralization of labile P_o is not affected by redox conditions. Substrate-induced P mineralization was slightly higher under flooded than drained conditions ($r^2 = 0.63$) (Fig. 2.8). Similarly, Newman and Reddy (1993) observed that enzymatic mineralization of P_o in hypereutrophic lake sediments is unaffected by short-term (< 24 h) anaerobic conditions. Racz (1979) observed equal to higher mineralization rates under anaerobic than aerobic conditions for organic soils. In contrast to our findings, Bridgham et al. (1998) observed that P mineralization was higher under anaerobic than aerobic conditions in sixteen northern wetlands. However, these researchers incubated their samples for 59 weeks that could have included mineralization of nonlabile P pools. D'Angelo and Reddy (1999) and DeBusk and Reddy (1998) observed higher rates of C mineralization in wetlands under aerobic than anaerobic conditions. Likewise, McLatchey and Reddy (1998) observed that ribonucleic acid (RNA)-P mineralization and substrate-induced respiration (SIR) rates are higher under aerobic than anaerobic conditions. While DeBusk and Reddy (1998) obtained a threefold increase in C mineralization between anaerobic and aerobic conditions for WCA-2A, this study did not show influence of O_2 availability on P_o mineralization at the same site. Therefore, short-term P_o mineralization in WCA-2A is independent of C mineralization and unaffected by O_2 availability.

Alkaline phosphatase activity was negatively correlated with SIPM for detritus during the dry season. In the detritus, $NaHCO_3$-extractable P_i during the dry season was negatively correlated ($r = -0.85$; $P<0.01$) with APA, suggesting that the level of $NaHCO_3$-

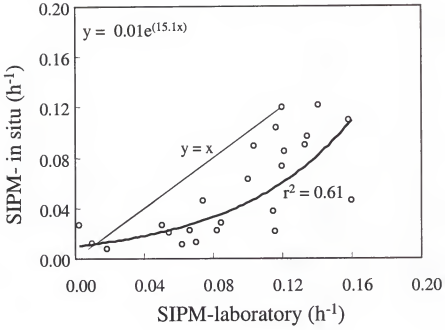

Fig. 2.7. Substrate-induced P mineralization for drained samples collected from WCA-2A, August 1996. n = 24

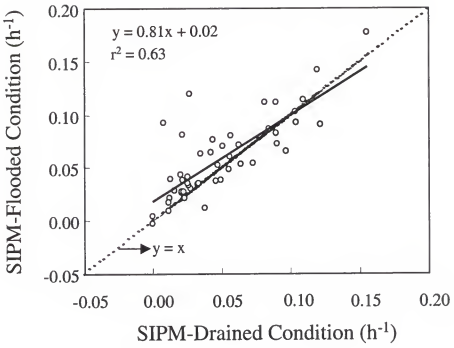

Fig. 2.8. Substrate-induced P mineralization (SIPM) under flooded and drained conditions. (n = 8 stations/depth; 3 depths; 2 seasons; 48 total)

extractable P_i, particularly within 5 km from the inflow - inhibits the activities of alkaline phosphatases. An inverse relationship exists between nutrient availability and enzyme activity (Sinsabaugh et al., 1993). In wetlands, Kang and Freeman (1999) showed that phosphatase activity increases when P_i concentrations are low but decreases when P_i concentrations are high. The SIPM rate for both 0-10 and 10-30 cm soils showed strong positive correlation ($P<0.01$) with APA during the dry season. It is likely that $NaHCO_3$-extractable P_i for these soils were low, and therefore, less inhibiting to phosphatase activity. No significant correlation, however, was obtained between SIPM and APA for the detritus and surface soils during the wet season. Likewise, Trasar-Cepeda and Carballas (1991) observed no correlation between phosphatase activity and net mineralization in Andosols. Higher moisture content during wet compared to dry seasons probably promoted the release of more labile P_o and mineralization becomes less governed by enzyme activity. Harrison (1982a) explained that the increase in moisture content from autumn to spring caused higher ^{32}P-RNA mineralization rate in spring than autumn. This researcher also observed increase in phosphatase activity with moisture content. Our result supported the observation that increase in rainfall decreases the amount of monoester-P and increases diester-P (Sumann et al., 1998). Diester-P are more available for enzymatic hydrolysis than monoester-P.

For the detritus, SIPM rate was negatively correlated ($P<0.01$) with mass $C:P_o$ during the dry season while no significant correlation was obtained during the wet season. For the 0-10 cm soil, SIPM was positively correlated with $C:P_o$ at the $P<0.05$ and $P<0.01$ levels during the dry and wet seasons, respectively. There was no significant correlation obtained for SIPM with mass $C:P_o$ ratio for the 10-30 cm soil during the dry

season, while a positive correlation ($P<0.05$) was obtained during the wet season. Hence, moisture affects substrate quality (as C:P_o) and, consequently, SIPM rates. Amador and Jones (1997) had shown that soil moisture effects on carbon mineralization for a Histosol were dependent on soil C:P ratio. These researchers observed decreases in rates of respiration with soil water content at C:P ratios of 200 and 20. Bridgham et al. (1998) observed that turnover rates were high in wetlands with low C:P values under anaerobic conditions while the opposite was found under aerobic condition. This indicates a dependency on substrate quality in terms of mass C:P ratio with redox conditions.

Except for the 0-10 cm soil during dry season, there were no significant relationships obtained between microbial biomass P and SIPM. Microbial biomass P in the 0-10 cm soil increased ($P<0.05$) with SIPM. Similarly, Saggar et al. (1998) obtained no significant correlation between microbial respiration and P mineralization in forest-floor soils. The $NaHCO_3$-extractable P_i was positively correlated ($P<0.05$) with SIPM in the detritus only during the dry season. Greater amounts of P_i in the detritus provided additional nutrient for microbial metabolism and, consequently, more mineralization of P_o. However, during the wet season, SIPM was negatively correlated ($P<0.05$) with $NaHCO_3$-P_i for the 0-10 cm depth while no significant correlation was obtained for the detritus and 10-30 cm soil. In twenty-five woodland surface soils, Harrison (1982b) obtained a positive correlation ($P<0.01$) of extractable-P_i with ^{32}P-RNA mineralization rate during the fall, while no correlation was obtained for the spring with the aforementioned parameters.

When SIPM rates for the dry season were converted to percentages of $(SIPM)_{max}$ for each depth, the detritus and 0-10 soil were correlated with distance at $P<0.01$ and $P<0.05$, respectively (Fig. 2.9). However, the detritus showed a negative correlation between SIPM rate as % $(SIPM)_{max}$ and distance, while the 0-10 cm soil showed a positive correlation during the dry season. There was no correlation observed for the 10-30 cm soil during dry season. For detritus, SIPM showed a negative correlation with distance probably because of a decrease in labile P_o with distance from the inflow. For the 0-10 cm soil, on the other hand, increases in APA (Wright and Reddy, 2000) and microbial efficiency (microbial biomass as percent of TP) with distance from the inflow resulted to an increase in SIPM with distance.

The SIPM rates for the 0-10 and 10-30 cm soils were positively correlated with distance at $P<0.01$ and $P<0.05$, respectively, while there was no significant correlation obtained for the detrital layer during the wet season. The increase in SIPM with distance for the 0-10 and 10-30 cm soils seemed due to the increase in microbial efficiency. The coefficients of variation for SIMP averaged across three stations were 82 and 66% in the detritus and 0-10 cm soil, respectively (Table 2.2). High coefficient of variations obtained for SIPM could be due to differences in substrate quality and enzyme activities. There are several factors known to influence the activities of enzymes. They are moisture, temperature, pH, presence of proteases, end-product inhibitors, etc (Speir and Ross, 1978).

Decrease in P_o mineralization with soil depth can be linked to decreases in the microbial biomass and labile P_o pools. There was no general relationship observed between P_o mineralization and P enrichment as P_o mineralization is being governed by a

group of soil factors that differ from one soil depth to the other. Among these parameters are enzyme activity (APA), moisture content, substrate quality (mass C:P or C:P_o ratios), inorganic P, labile P_o content, redox status, and temperature. Some factors are also interrelated in regulating P_o mineralization. For example, high amounts of inorganic P particularly in the detritus, inhibits the activity of alkaline phosphatase; more labile P_o becomes available with increased in moisture content; and the influence of redox on P_o mineralization is dependent on nature of the substrate. These interrelationships among parameters obscured the effect of P enrichment on P_o mineralization.

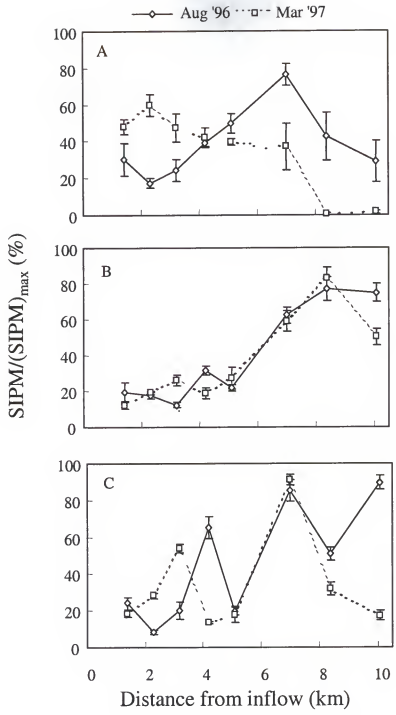

Fig. 2.9. Substrate-induced P mineralization (SIPM)/$(SIPM)_{max}$ of (a) detritus, (b) 0-10 cm soil, and (c) 10-30 cm soil during the wet (August 1996) and dry (March 1997) seasons.

Conclusions

This study revealed that external P loading to WCA-2A had resulted to increases in most P pools. Among these pools - microbial P and labile P_o - comprised substantial portions of the total P. The microbial biomass involved in transforming organic to inorganic P, had a rapid turnover of 13 d. The microbial pools in areas not affected by external P loading although small in size were efficient in assimilating the meager amount of inorganic P present in the system. The labile P_o pool had a turnover time comparable to that of microbial biomass and decreased with soil depth.

Potentially mineralizable-P provided estimates of turnover rates of various P pools that could be useful to wetland scientists determining the duration at which an impacted wetland can be brought to a desirable or pristine condition when P loading has been abated. Correlations obtained for microbial biomass, labile P_o, total P, and substrate quality (as mass C:P_o ratio) with PMP revealed the significance of these factors in regulating P_o mineralization. For phosphatase activity, several biogeochemical factors such as inorganic P, temperature, and moisture, confounded the relationship that may have been obtained between P_o mineralization and APA.

Results obtained for SIPM showed that aeration does not affect P_o mineralization. Decrease in SIPM with increase in APA for detritus revealed the inhibitory effect of high levels of P_i to phosphatase activity. Increase in SIPM with decrease in P for soils, on the one hand, indicated the importance of microbial efficiency that increases when P_i is limited. Decreases in both PMP and SIPM with soil depth were associated to decreases in the microbial biomass and labile P_o pools. Thus, the microbial biomass and labile P_o pools play significant roles in P_o mineralization.

Factors governing P_o mineralization can be integrated into a model that can guide decision makers to make sound judgment for problems related to wetland eutrophication. The predictive capability of a model for a particular pathway such as mineralization-immobilization, however, would depend largely on the accuracy and sensitivity of the inputs. Thus, there is a need to determine the significance of soil factors investigated using several wetland types in order to understand P_o mineralization on a broader scale.

CHAPTER 3

ROLE OF INORGANIC ELECTRON ACCEPTORS ON ORGANIC PHOSPHORUS MINERALIZATION IN DETRITUS AND SOILS OF THE FLORIDA EVERGLADES

Introduction

The transformation of nutrients or contaminants in wetlands is controlled by the presence of both aerobic and anaerobic zones. Upon flooding, three distinct soil layers are formed: (i) an oxidized or aerobic layer where oxygen is present; (ii) a zone of alternate aerobic and anaerobic conditions due to water table fluctuations; and (iii) a reduced layer where oxygen is absent at all times. The supply of O_2 may be limiting in wetlands high in organic C; thus, the microbial population must utilize alternate electron acceptors to meet its metabolic needs. Electron acceptors are reduced sequentially as follows: $O_2 > NO_3^- > Mn^{+4} > Fe^{+3} > SO_4^{-2} > HCO_3^-$. This sequence conforms to thermodynamic theory with the electron acceptor having the higher redox potential being reduced first (Zehnder and Stumm, 1988).

In wetlands, a major portion of the P is present as organic P (P_o). Thus, P released through decomposition of organic matter can regulate productivity as well as water quality (Reddy and D'Angelo, 1994). Decomposition in wetlands is often electron-acceptor-limited compared to terrestrial ecosystems (Koch-Rose et al., 1994; DeBusk and Reddy, 1998). Net movement of P from detritus to the microbial pool and eventually to

the soluble pool is dependent not only upon the quantity and quality of organic matter but also upon the prevailing microbial respiration. With each type of microbial respiration, the rate of organic matter decomposition will depend on bacterial efficiency and the capacity of the soil to supply electron acceptors (Reddy et. al, 1986). Demand for and supply of O_2 regulates the balance between aerobic and anaerobic conditions, with anaerobic respiration prevailing when dissolved O_2 concentrations fall below 0.003 mM (Smith and Arah, 1986).

Oxygen serves not only as a terminal electron acceptor during organic matter degradation but also as a reactant in the initial reaction involving oxygenase catalysis on the substrate molecules (Kristensen et al., 1995). The C source and amount of energy present influence the type and metabolic rates at any given depth in the soil profile (Fenchel and Blackburn, 1979; Sörensen et al., 1979). Several types of microbial metabolic functions can occur simultaneously in the soil profile when substrates are non-limiting and competition among electron acceptors is absent (Reddy et. al, 1986).

The mineralization of P_o via microorganisms occurs either directly through extracellular and periplasmic hydrolases or indirectly through changes in ionic composition, pH, redox, and other conditions that influence the tenacity with which P is held onto organic matter (Wetzel, 1999). The solubilization of metals such as Fe and Mn after flooding can either reduce (Pulford and Tabatabai, 1988; Freeman et al., 1996) or increase (Pulford and Tabatabai, 1988) enzymatic activities that affect the mineralization of organic matter.

Redox conditions are also influenced by hydrologic conditions. Flooding alters the redox condition, which influences the rate at which P is released from organic matter.

McLatchey and Reddy (1998) observed that the ribonucleic acid (RNA)-P mineralization rate of surface organic soils under aerobic conditions was four times higher than under anaerobic conditions. Higher P release under flooded compared to drained conditions appears due to the solubilization of organic matter during anaerobic decomposition (Mahapatra and Patrick, 1969; Reddy, 1983). In lakes, Reddy and Ivanoff (1991) showed that a continuous supply of NO_3^- and SO_4^{-2} in the water column, and the mixing of sediments with oxygenated overlying water, resulted in higher rates of organic matter decomposition than under strictly anaerobic conditions.

The Florida Everglades, a formerly contiguous wetland, has been divided into hydrological units by a system of canals and levees. The hydrological units, known as water conservation areas (WCAs), were established for flood control, water supply, and wildlife habitat. At present, water flows from one hydrological unit to the other through canals, pump stations, and water control structures that are regulated by predefined water schedules. Nutrient loading to WCA-2A from external sources has resulted in changes in macrophyte communities as well as soil nutrient storage. Increased productivity resulting from nutrient loading has increased P storage in the organic pool (Koch-Rose et al. 1994; Qualls and Richardson, 1995; Reddy et al., 1998). Seasonal changes in hydrology for some areas of the Everglades can alter the redox status and ultimately affect nutrient regeneration from organic matter. The Everglades Histosols can either be highly reduced or oxidized due to water level fluctuations and low amounts of inorganic electron acceptors to poise the redox conditions (Koch-Rose et al., 1994).

The objectives of this study were to determine: (i) the rate of labile P_o mineralization in the presence of various electron acceptors, and (ii) the relationships

among factors such as alkaline phosphatase activity (APA), microbial biomass P, substrate C:P_o ratio and labile P_o with respect to P_o mineralization in the presence of each inorganic electron acceptor.

Materials and Methods

Study Area

Water Conservation Area - 2A (WCA-2A), a 447 km^2 marsh area of the northern Everglades, is bounded on the northeast by the Hillsboro Canal and on the southwest by the North New River Canal (Fig. 3.1). About 58% of water entering WCA-2 originates from the EAA (South Florida Water Management District, 1992). Surface water flows to WCA-2A through four S-10 control structures located along the Hillsboro Canal, and through pump station S-7 on the North New River Canal. About 53% of the total P loading to WCA-2A enters through S-10 structures annually, while 25 and 22% of the total P loading enters through S-7 pump station and through direct rainfall, respectively (South Florida Water Management District, 1992). Over the past three decades, a shift in vegetation from sawgrass (*Cladium jamaicense* Crantz) to cattail (*Typha* spp.) close to the water inflow structures has been coincident with the drainage of nutrient-laden surface waters from the adjacent Everglades Agricultural Area (EAA) into WCA-2A.

The study transect traverses the marsh for approximately 10 km, from the S-10 water inflow control structure moving southward, with vegetation shifting concurrently from cattail to sawgrass. Eight sampling stations at 1.4, 2.3, 3.2, 4.2, 5.1, 7, 8.4, and 10.1 km from the inflow structure were established along this transect (Fig. 3.1).

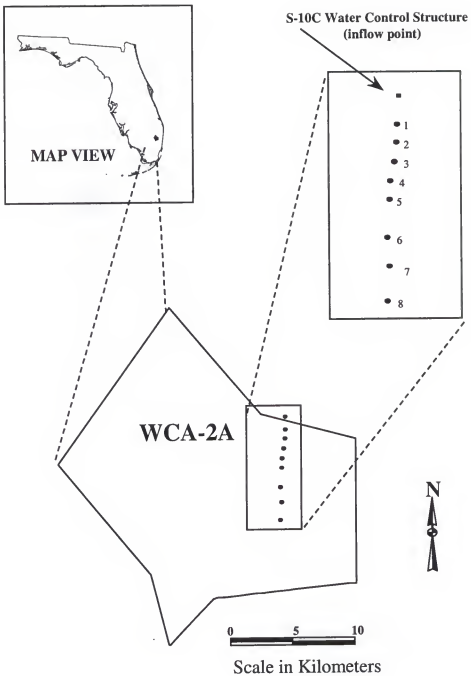

Fig. 3.1. Map showing sampling locations along a eutrophic gradient in Water Conservation Area-2A of the Everglades.

Sample Collection

Detritus and soil samples were collected from eight stations during the dry season in March 1997. Plant detritus was hand-picked from the surface before a 5-cm diameter polyvinyl core was pushed into the soil to a depth of 30 cm. Four cores were collected from each station and each core was sectioned into 0-10 and 10-30 cm depths. All four sections of the same depth from each station were combined and then mixed thoroughly before a representative subsample was taken for future analysis. The subsamples were kept on ice and transported to the laboratory for storage at 4°C in polyethylene containers.

Potentially Mineralizable-P (PMP)

Anaerobic Incubation:

Discernible live roots and shells were removed from the detritus or soil samples before homogenization with a food processor. A 0.5 g dry-equivalent of wet sample was weighed into 60-mL serum bottles in three sets of triplicates for each anaerobic treatment (i.e. NO_3^--, SO_4^{-2}-, or HCO_3^--reducing). Two mL of deionized distilled water was added to promote uniformity throughout the bottom of each bottle, and the bottle was capped with a rubber septum and covered with aluminum. Each bottle was evacuated for 10 s using 100-kPa suction and purged with N_2 gas for 3-4 min to displace any trapped air. The bottles then were incubated at 30°C. For moisture content, a 10-g subsample was dried in a forced-draft oven for 72 h at 70°C.

Sealed serum bottles were sampled at random and analyzed for CH_4 gas using a gas chromatograph equipped with a thermal conductivity detector (TCD). Gas chromatograph settings were as follows: 1.8 m by 2 mm i.d. stainless steel column with Poropak N (80/100) (Supelco; Bellefonte, PA); helium flow rate of 20 mL min^{-1}; and injector, column, and detector temperatures of 100, 50, and 100°C, respectively. Bottles were incubated at 30°C and the electron acceptor was added as soon as CH_4 appeared. Solutions containing nitrate or sulfate and deionized distilled water were purged with N_2 gas for 10 min before being added to the designated bottles. Three sets of triplicates representing nitrate-reducing conditions received 0.5 mL of 1.6 mg N mL^{-1} (KNO_3); another three sets representing sulfate-reducing conditions received 0.5 mL of 2.3 mg S per mL (K_2SO_4); and the remaining three sets representing HCO_3^- -reducing conditions received 0.5 mL of distilled water. One set of each treatment (NO_3^--, SO_4^{-2}-, and HCO_3^--reducing) as a control was quantitatively transferred to 50-mL centrifuge tubes and immediately equilibrated with 25 mL of 0.5 M $NaHCO_3$ (pH = 8.5 ± 0.1) for 30 min. The tubes were centrifuged at 4066 × *g* for 10 min and the supernatant was passed through a 0.45 µm membrane filter. The residue was subsequently equilibrated with 25 mL of 1 M HCl for 3 h and then was centrifuged and filtered as described previously. The remaining two sets of each treatment were incubated at 30°C for 15 d. After incubation, one set of each treatment was extracted in the same manner as for the control samples.

Potential mineralizable-P excluding any precipitation of mineralized P from P_o was calculated as follows:

$$PMP = [(NaHCO_3\text{-}P_i)_{t=15} - (NaHCO_3\text{-}P_i)_{t=0}]/t$$

where $(NaHCO_3\text{-}P_i)_{t=15}$ is bicarbonate-extractable inorganic P after the 15-d incubation; $(NaHCO_3\text{-}P_i)_{t=0}$ is bicarbonate-extractable inorganic P at the onset of incubation; t is the length of incubation in d; and PMP is the potentially mineralizable-P rate in mg kg^{-1} d^{-1}.

To account for precipitation of P mineralized from P_o, potentially mineralizable-P was calculated as follows:

$$PMP = \{[(NaHCO_3\text{-}P_i)_{t=15} + (HCl\text{-}P_i)_{t=15}] - [(NaHCO_3\text{-}P_i)_{t=0} + (HCl\text{-}P_i)_{t=0}]\}/t$$

where $(NaHCO_3\text{-}P_i)_{t=15}$ and $(HCl\text{-}P_i)_{t=15}$ are bicarbonate-extractable inorganic P and acid-extractable inorganic P, respectively, after the 15-d incubation; $(NaHCO_3\text{-}P_i)_{t=0}$ and $(HCl\text{-}P_i)_{t=0}$ are bicarbonate-extractable inorganic P and acid-extractable inorganic P, respectively, at the onset of incubation; t is the length of incubation in d; and PMP is the potentially mineralizable-P rate in mg kg^{-1} d^{-1}.

Aerobic Incubation:

To maintain aerobic conditions, a portion of the homogenized wet detritus/soil sample was spread thinly on a 20-cm × 28-cm Gelman glass fiber A/C filter, overlying paper towels for 30 min to drain excess water. The moisture content of the drained detritus/soil sample was determined as described previously.

A 0.5-g dry equivalent of drained sample was weighed into a tared plastic weighing boat in two sets of triplicates. Drained samples representing the O_2-reducing treatment were spread thinly on the entire bottom of the boat to allow O_2 exchange. The weighing boats were arranged at random in a 20-cm × 28-cm rack that was later covered with polyethylene plastic to protect samples from condensate. The rack was set in a 46-cm-high plastic container filled with distilled water to about 20 cm. Water was added to the container to prevent the sample from drying during incubation. The container was

covered loosely to permit air exchange and incubated at 30°C for 15 d. After incubation, samples were transferred quantitatively to 50-mL centrifuge tubes and equilibrated with 25 mL of 0.5 M $NaHCO_3$ (pH = 8.5 ± 0.1) for 30 min with end-to-end shaking at low speed. Tubes were centrifuged at 4066 × *g* for 10 min and filtered through 0.45-μm membrane filters using 100 kPa suction. The residue was equilibrated with 25 mL of 1M HCl for 3 h and filtered in the same fashion as with 0.5 M $NaHCO_3$.

Drained samples used as controls were weighed into 50-mL centrifuge tubes in triplicate and samples were immediately equilibrated with 0.5 M $NaHCO_3$ (pH = 8.5 ± 0.1) followed by 1 M HCl as described previously. Potentially mineralizable-P with and without correction due to precipitation was calculated the same as for the flooded samples.

Substrate-induced P Mineralization (SIPM)

The remaining set (previously incubated with electron acceptors for 15 d) of each treatment was analyzed for SIPM. A half mL of 200 mg P L^{-1} as glucose-6-phosphate (disodium salt, Sigma Chemical Co., St. Louis, MO) was added to each sample before incubation at 30°C for 4 h. After incubation, samples were extracted in the same fashion as described previously.

Substrate-induced P mineralization excluding any precipitation of mineralized P from P_o was calculated as follows:

$$SIPM = [(NaHCO_3\text{-}P_i)_{t=4}] - [(NaHCO_3\text{-}P_i)_{t=0}]/ t$$

where $(NaHCO_3\text{-}P_i)_{t=4}$ is bicarbonate-extractable inorganic P after the 4-h incubation, mg kg^{-1}; $(NaHCO_3\text{-}P_i)_{t=0}$ is bicarbonate-extractable inorganic P at the beginning of

incubation, mg kg^{-1}; t is the incubation time, h; and SIPM is the substrate-induced P mineralization rate, mg kg^{-1} h^{-1}.

Substrate-induced P mineralization accounting for any precipitation of mineralized P from P_o was calculated as follows:

$$SIPM = \{[(NaHCO_3\text{-}P_i)_{t=4} + (HCl\text{-}P_i)_{t=4}] - [(NaHCO_3\text{-}P_i)_{t=0} + (HCl\text{-}P_i)_{t=0}]\}/t$$

where $(NaHCO_3\text{-}P_i)_{t=4}$ and $(HCl\text{-}P_i)_{t=4}$ are bicarbonate-extractable inorganic P and acid-extractable inorganic P after the 4-h incubation, respectively; $(NaHCO_3\text{-}P_i)_{t=0}$ and $(HCl\text{-}P_i)_{t=0}$ are bicarbonate-extractable inorganic P and acid-extractable inorganic P at the onset of incubation, respectively; t is the length of incubation in h; and SIPM is the substrate-induced P mineralization rate in mg kg^{-1} h^{-1}.

Microbial biomass P

A 0.5-g dry equivalent of moist detritus/soil was weighed into serum bottles in six sets of triplicates. Two mL of deionized distilled water was added to each bottle before it was capped with a rubber septum and covered with aluminum. Capped bottles were evacuated for 10 s using 100-kPa suction and purged with O_2-free N_2 gas for 3-4 min. Bottles were incubated at 30°C and treated with the designated electron acceptor as soon as CH_4 appeared. Nitrate and sulfate solutions and deionized distilled water were bubbled with N_2 gas for 10 min before added to the designated bottles. Two sets of triplicates representing nitrate-reducing conditions received 0.5 mL of 1.6 mg N mL^{-1} (KNO_3) per bottle, another two sets representing sulfate-reducing conditions received 0.5 mL of 2.3 mg S per mL (K_2SO_4), and the remaining two sets representing methanogenic conditions received 0.5 mL of P-free distilled water per bottle. Bottles were incubated for 15 d at 30°C.

A 0.5-g dry weight equivalent of drained detritus/soil was weighed using plastic weighing boats into two sets of triplicates. Drained samples representing O_2-reducing treatment were spread thinly on the entire bottom of the boat to allow O_2 exchange. The filled boats were arranged at random in a 20-cm × 28-cm rack that was later covered with a polyethylene plastic to protect samples from condensate. The rack was set in a 46-cm-high plastic container filled with distilled water to about the 20-cm depth. The container was covered loosely and kept in the dark at 30°C for 15 d.

After incubation, triplicates of each treatment were transferred quantitatively to 50-mL centrifuge tubes using 25 mL of 0.5 M $NaHCO_3$ (pH = 8.5) and were equilibrated for 16 h using an end-to-end shaker set at low speed. Tubes were centrifuged at 4066 *g* for 10 min and supernatant was passed through a 0.45-μm membrane filter (Gelman Sciences, Ann Arbor, MI). The remaining triplicates of treatments (NO_3^--, SO_4^{-2}-, or HCO_3-reducing) were treated with 1 mL of ethanol-free $CHCl_3$ for 24 h and triplicates from the O_2-reducing treatment were transferred to 50-mL centrifuge tubes before the addition of $CHCl_3$. Bottles/tubes were uncapped for 24 h to remove $CHCl_3$. Samples in serum bottles were transferred quantitatively to 50-mL centrifuge tubes and equilibrated with 0.5 M $NaHCO_3$ (pH = 8.5) as described previously. Likewise, tubes designated for O_2-reducing treatment were equilibrated with 0.5 M $NaHCO_3$ (pH = 8.5). A 5-mL aliquot of the $NaHCO_3$ extracts was analyzed for total P after a $K_2S_2O_8$ oxidation followed by H_2SO_4 digestion (Bowman, 1989). Microbial biomass P was calculated as the difference in total P for the $CHCl_3$-treated and control samples, and was reported uncorrected for efficiency. Using a correction factor determined by previous researchers can cause overestimation of microbial biomass P (Chapter 2).

Effect of Nitrate Concentration on Organic P Mineralization

The PMP and SIPM rates were determined on detritus and 0-10 cm soil at two different levels of nitrate-N addition. Detritus and 0-10 cm soil collected 1.4, 4.2, 7.0, and 10.1 km from the water inflow point at S10-C were used for this section.

Triplicates of 0.5-g dry equivalent of homogenized detritus or soil were weighed into 50-mL centrifuge tubes as non-incubated controls and were amended with 2 mL of deionized distilled water. The samples were immediately equilibrated with 25 mL of 0.5 M $NaHCO_3$ (pH=8.5±0.1) for 30 min. The tubes were centrifuged at 4066 *g* for 10 min before supernatant was passed through a 0.45 µm membrane filter. The residue was equilibrated with 25 mL of 1 M HCl for 3 h. The tube was centrifuged and supernatant was filtered as described earlier.

Four sets of 0.5-g dry equivalent of moist detritus or soil were weighed into 60-mL serum bottles. Triplicates of each set were weighed. Two mL of deionized distilled water was added into each bottle before it was capped and sealed. Bottles were evacuated for 10 sec using 100 kPa suction and purged with N_2 gas for 10 min. Four sets were divided into two sets with 0.5 mL of deoxygenated nitrate (1.6 mg N mL^{-1}) solution added per bottle before incubation and the other two sets with two 0.5 mL of the same nitrate solution added at the beginning and middle of incubation. All sets were incubated at 30°C for 15 d. After incubation, one set of each treatment was equilibrated with 0.5 M $NaHCO_3$ followed by 1 M HCl as described previously. A half mL of 200 mg P L^{-1} as glucose-6-phosphate was added to the second set and incubated for another 4 h at 30°C. After incubation, samples were equilibrated as described previously. Potentially mineralizable-P and SIPM were calculated as described previously.

Chemical Analyses

Soluble reactive P (SRP) in the 0.5 M $NaHCO_3$ extract was analyzed by the Murphy and Riley (1962) method at 880 nm using an ultraviolet-visible spectrophotometer, while SRP in the 1 M HCl extracts was analyzed using an automated ascorbic acid procedure (USEPA, 1993).

Statistical Analyses

Potentially mineralizable-P and SIPM were described by zero-order kinetics and reported as mg kg^{-1} d^{-1} and mg kg^{-1} h^{-1}, respectively. The PMP and SIPM rates by depth were compared among electron acceptor treatments using PROC GLM (SAS version 6.12, 1996). Similarly, PMP and SIPM by depth were differentiated among stations using PROC GLM. Means were separated using least significant difference (LSD) by depth. Linear regression analyses among variables were generated using Microsoft Excel Version 7.0.

Results and Discussion

Potentially Mineralizable-P (PMP)

The PMP rates calculated without correction for the possible precipitation of P_i from mineralized P_o underestimated PMP rates measured as net changes in combined $NaHCO_3$- and HCl-extractable P_i (Fig. 3.2). A ten-day incubation at 40°C yielded the same observation (Chapter 2). It is likely that, with the longer incubation time and substantial amounts of Ca present in detritus and soils of WCA-2A, a significant portion of the mineralized P from P_o was precipitated as calcium P (DeBusk et al., 1994).

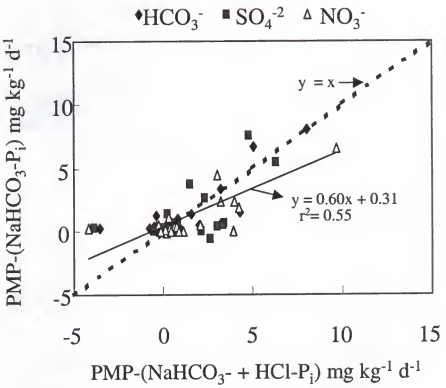

Fig. 3.2. Potential mineralizable P (excluding any precipitation) as a function of PMP measured as net increase from combined $NaHCO_3$- and HCl-extractable-P_i in anaerobic treatments. (3 depths; 3 electron acceptor types; 8 stations; n = 72).

In the following discussion, all PMP rates are those corrected for precipitation in which net changes of combined $NaHCO_3$- and HCl-extractable P are considered.

The detritus had higher PMP rates than the 0-10 and 10-30 cm soils (Table 3.1). This was anticipated because concentrations of pore water and extractable-P_i are higher in the detritus than in surface soils (Koch-Rose et. al., 1994; Reddy et. al., 1998). High amounts of P_i, particularly at the impacted/enriched sites, can be nourishment for microbial metabolism. This was also evident from the higher microbial biomass P concentration in detritus compared to the 0-10 and 10-30 cm soils. Because the amount of labile P_o substrate decreased with soil depth, it is likely that P_o mineralization would also decrease with soil depth. In detritus, there was no significant difference of PMP among impacted, transitional, and non-impacted stations. The difference among stations probably became less apparent for the detritus samples that have high labile P_o substrate concentrations. The PMP, however, was significantly different ($P<0.01$) among electron acceptors. Although similar in magnitude, the PMP for detritus under anaerobic conditions (i.e. NO_3^-, SO_4^{-2}, or HCO_3^- as electron acceptor) revealed net mineralization while PMP under aerobic conditions indicated net immobilization. It is likely that P_i released from P_o mineralization is immediately immobilized; hence, mineralization becoming less apparent. Thus, PMP for detritus was higher ($P<0.01$) under anaerobic than under aerobic conditions. The PMP values under NO_3^--, SO_4^{-2}-, or HCO_3-reducing conditions, however, are not significantly different from each other. Similarly, D'Angelo and Reddy (1999) observed that organic C mineralization of wetland soils under NO_3^--, SO_4^{-2}-, or HCO_3^--reducing conditions are not different from each other. Reddy and Ivanoff (1991), using *p*-nitrophenyl phosphate with surface lake sediments and Bridgham

et al. (1998) for northern wetlands, observed higher P_o mineralization under anaerobic than aerobic conditions. The higher energy yield under aerobic than anaerobic conditions increased the nutrient demand of the microorganisms, thus immobilizing more P_i (Scothorst, 1977) and resulting in less apparent P_o mineralization. Meanwhile, some reduced metal ions released under anaerobic conditions can activate phosphatases (Juma and Tabatabai, 1977; Pulford and Tabatabai, 1988). In contrast, Bridgham and Richardson (1992) and De Busk and Reddy (1998) observed that microbial respiration in peat soils was higher under aerobic than anaerobic conditions.

For the 0-10 cm soil, the PMP among stations was significantly different ($P<0.05$). Potentially mineralizable-P in the impacted and transitional stations was higher than in the non-impacted station. The type of electron acceptor, however, had no effect on PMP for the 0-10 cm soil. It is probable that the labile P_o substrates at this depth consisted of those unaffected by aeration. Reddy and Ivanoff (1991) found no significant difference between aerobic and anaerobic incubations when glucose-6-phosphate or glycerophosphate was added to surface lake sediments. McLatchey and Reddy (1998), on the one hand, observed that aerobic hydrolysis of ribonucleic acid-P (RNA-P) was four-and eighteen-fold higher than under NO_3^-- and HCO_3^--reducing conditions, respectively. These researchers measured hydrolysis rates after a 24-h incubation of soil slurries that had reached steady state Eh. It seems that both the type of electron acceptor and the nature of substrate regulate P_o mineralization. It is, however, difficult to generalize at this point because of differences in incubation time and experimental set-up. McLatchey and Reddy (1998) used soil slurries from stirred reactors that tend to provide more aeration than the drained incubations used for this

study, and microorganisms can be sensitive to agitation. Dannenberg et al. (1997) observed higher CH_4 production when anoxic paddy soils were incubated without agitation than with shaking or stirring.

For the 10-30 cm soils, there was no significant difference among electron acceptors and among stations (Table 3.1). There was no significant difference obtained among electron acceptors and stations for the 10-30 cm soil because labile P_o substrates at this depth may be limiting P_o mineralization.

Alkaline phosphatase activity (Wright and Reddy, 2000) showed no correlation with PMP while microbial biomass P demonstrated significant ($P<0.05$) correlations with PMP in the presence of various electron acceptors. It is likely that alkaline phosphatase enzymes are regulated by several environmental factors that may have obscured relationships that can be established with P_o mineralization. For woodland soils, Harrison (1982b) obtained no correlation of phosphatase activity with ^{32}P-RNA mineralization although phosphatase activity was related to soil respiration. Alkaline phosphatase activity, measured using *p*-nitrophenyl phosphate as a substrate, did not correlate with P_o mineralization, probably because other forms of P_o are controlling P mineralization. It is also probable that, when electron acceptors are non-limiting, microbial activity and APA may not be related.

Table 3.1. Potentially mineralizable-P as affected by added electron acceptor among stations in WCA-2A.

Stations	HCO_3^-	SO_4^{-2}†	NO_3^-‡	O_2
	----------mg kg^{-1} d^{-1}----------			
			detritus	
Impacted§	5.81	4.21	5.58	-6.40
Transitional¶	2.03	2.82	2.58	-4.14
Non-impacted#	0.75	1.37	3.01	-0.45
Mean±SE	2.86±1.52a	2.80±0.82a	3.72±0.94a	-3.66±1.73b
			0-10 cm soil	
Impacted	0.05	0.46	0.47	0.72
Transitional	0.07	2.32	0.33	-2.43
Non-impacted	-1.72	-1.79	-2.00	-1.36
Mean±SE	-0.53±0.59a	0.33±1.19a	-0.40±0.80a	-1.02±0.92a
			10-30 cm soil	
Impacted	0.01	0.11	0.20	-0.70
Transitional	0.08	0.26	0.13	-0.62
Non-impacted	0.33	-0.08	0.02	1.30
Mean±SE	0.14±0.10a	0.10±0.10a	0.12±0.05a	-0.01±0.66a

† added as 1.15 mg S/bottle as K_2SO_4
‡ added as 0.8 mg N/bottle as KNO_3
§ stations 1.4 to 3.2 km from the inflow
¶ stations 4.2 to 7.0 km from the inflow
# stations 8.4 to 10.1 km from the inflow
SE = standard error; Values with the same letter within a row are not significantly different at $P<0.05$.

Potentially mineralizable-P was positively ($P<0.01$) correlated with microbial biomass P under NO_3^--, SO_4^{-2}- and HCO_3^--reducing conditions, while it was negatively ($P<0.01$) correlated under aerobic conditions. Likewise, PMP under reducing conditions was positively correlated ($P<0.01$) with total labile P_o while these parameters were negatively correlated ($P<0.01$) under O_2-reducing conditions. Microorganisms store available P_i from mineralized P_o under aerobic conditions; hence, microbial biomass is increased as more P_i is assimilated. Under aerobic and nutrient non-limiting conditions, microorganisms tend to store excess P in the form of polyphosphates (Gächter and Meyer, 1993). Facultative to obligate anaerobes, having lower growth yield than aerobes, would degrade labile P_o to P_i in order to supply their metabolic functions.

From Fig. 3.3, the phosphorus pools - microbial biomass, total labile P_o, and total P_o - in detritus and soils had turnover times of 62, 100, and 370 d, respectively when incubated at 30°C. These fractions had corresponding turnover rates of 13, 16, and 72 d at 40°C (Chapter 2). Joergensen et al. (1990) estimated that the turnover of the microbial biomass pool were 139, 62, and 4 d with incubation temperatures of 15, 25, and 35°C, respectively. While these researchers observed a fifteen-fold decrease in turnover when incubated from 25 to 35°C, the turnover of microbial biomass P in soils and detritus of WCA-2A decreased by five-fold with a 10°C increase in temperature. The microbial community in WCA-2A, a subtropical wetland, is probably less sensitive to elevated temperature.

The mass C:P_o ratio was not significantly correlated with PMP in the presence of any electron acceptor. Organic C as an electron donor was probably non-limiting, which may have concealed the effect of C:P_o ratio on P_o mineralization. In comparison,

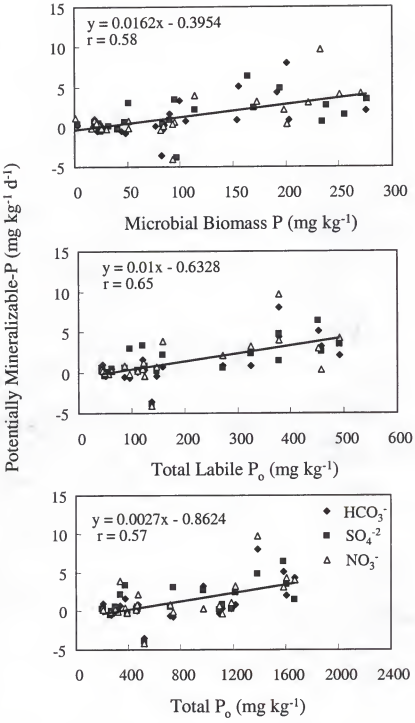

Fig. 3.3. Potential mineralizable P in detritus and soils of WCA-2A as a function of (a) microbial biomass P, (b) total labile P_o, and (c) total P_o.

Amador and Jones (1997) indicated that soil moisture effects on carbon mineralization for a Histosol were dependent on soil C:P ratio. These researchers observed decreases in rates of respiration with soil water content at C:P ratios of 200 and 20.

Substrate-induced P Mineralization (SIPM)

The SIPM technique uses labile P_o such as glucose-6-P to determine the ambient microbial and enzyme activities. This is similar to substrate-induced respiration (SIR) a measure of catabolic rate processes that can be related to actively metabolizing biomass or microbial respiration (Anderson and Domsch, 1978a). The mineralization of an easily degradable substrate is carried out by two groups of microorganisms – those which exhibit an exponential growth in response to added substrate, and those that increase respiration rate without significantly increases in number (Stenström et al., 1998).

To determine if a considerable amount of P_i from mineralized P_o was precipitated after a 4-h incubation, the net change in P_i extracted from with $NaHCO_3$ and HCl was plotted against net change in $NaHCO_3$- extractable P_i (Fig. 3.4). For detritus and soils, the two fractions combined and the $NaHCO_3$-P_i fraction alone were significantly and linearly related ($r^2 = 0.81$) with a slope of 0.84. Thus, the precipitation of P_i from mineralized P_o is of minor importance when detritus and soils from a Ca-dominated system such as the Florida Everglades are subjected to short-term incubation.

The SIPM was higher for detritus than for 0-10 and 10-30 cm soils (Table 3.2). For detritus, there was no significant difference in SIPM among electron acceptors and stations. For the 0-10 cm soil depth increment, there were significant differences among electron acceptors ($P<0.01$) but not among stations. The SIPM was higher under O_2-

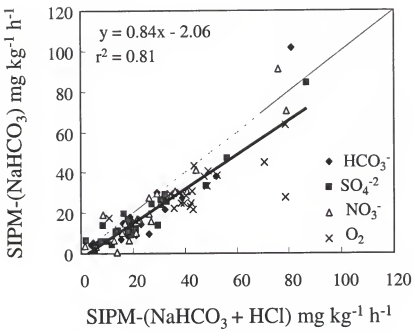

Fig. 3.4. Substrate-induced P mineralization (excluding any precipitation) as a function of SIPM measured as net increase from combined $NaHCO_3$-and HCl-extractable-P_i in detritus and soils previously incubated with various inorganic electron acceptors. Dashed line denotes the relationship y = x.

Table 3.2. Substrate-induced phosphorus mineralization (SIPM) as affected by added electron acceptor among plant communities in WCA-2A.

Stations	HCO_3^-	$SO_4^{-2\dagger}$	$NO_3^{-\ddagger}$	O_2
	----------mg kg^{-1} h^{-1}----------			
			detritus	
Impacted[§]	39	42	35	70
Transitional[¶]	30	30	32	43
Non-impacted[#]	36	41	35	37
Mean±SE	35±3a	37±4a	34±1a	50±10a
			0-10 cm soil	
Impacted	12	11	10	41
Transitional	14	7	19	38
Non-impacted	26	26	31	44
Mean±SE	17±5b	14±6b	20±6b	41±2a
			10-30 cm soil	
Impacted	15	14	23	38
Transitional	24	23	26	42
Non-impacted	18	18	32	38
Mean±SE	19±3c	19±3c	27±2b	39±1a

† added as 1.15 mg S/bottle as K_2SO_4

‡ added as 0.8 mg N/bottle as KNO_3

§ stations 1.4 to 3.2 km from the inflow

¶ stations 4.2 to 7.0 km from the inflow

stations 8.4 to 10.1 km from the inflow

SE = standard error; Values with the same letter within a row are not significantly different at $P<0.05$.

reducing than anaerobic conditions. For the 10-30 cm soil, there were significant differences in SIPM among electron acceptors ($P<0.01$) and stations ($P<0.05$). The rate of SIPM for 10-30 cm soil in the transitional station was higher than either impacted or non-impacted stations. Substrate-induced P mineralization in the 10-30 cm soil decreased in the order: $O_2>NO_3^->SO_4^{-2} = HCO_3^-$. Thus, the conclusion that aerobic metabolism is more efficient than anaerobic metabolism is verified for the 0-10 and 10-30 cm soils (Stanier et. al., 1977). This was, however, not revealed during a 15-d incubation. Under long-term incubation, the type of electron acceptor probably regulates P_o mineralization as well as its supply and consumption. It is also probable that P_o mineralization under long-term incubation is governed by different kinetics. McLatchey and Reddy (1998) observed that P mineralization in surface organic soil was four to eighteen times higher under aerobic than anaerobic conditions, and decreased with decrease in redox potential. The 0-10 and 10-30 cm soils from this study demonstrated only two- to three-fold increase in P mineralization under aerobic than anaerobic conditions, which suggests differential responses among P_o substrate with electron acceptor. This study used glucose-6-phosphate, while McLatchey and Reddy (1998) used RNA-P. Although organic matter decomposition is enhanced when soil Eh is increased to >300 mV, it is also worth considering the type/quality of P_o substrate when determining the rate of P_o mineralization (Koch-Rose et al., 1994).

There was a positive correlation ($P<0.01$) between APA (Wright and Reddy, 2000) and SIPM rates measured under NO_3^--, SO_4^{-2}-, and HCO_3^--reducing conditions, while APA was not correlated with SIPM under aerobic conditions. Oxygen, having a higher energy growth yield compared with other electron acceptors, probably does not

require the catalytic activity of phosphatases during the conversion of P_o to P_i. The lower growth yield of anaerobic compared to aerobic metabolism, on the other hand, increases the demand for phosphatase enzymes capable of mineralizing P_o to P_i.

Microbial biomass was positively ($P<0.05$) correlated with SIPM under O_2- and SO_4^{-2}- reducing conditions, while there was no correlation under NO_3^-- or HCO_3^--reducing conditions. The redox potential under NO_3-reducing conditions probably was too low to allow the conversion of NO_3^- to reduced species. It is also likely that SIPM under NO_3^--reducing conditions follows first-order rather than zero-order kinetics, and hence is dependent on electron-acceptor concentration. Drake et al. (1996) observed higher numbers of anaerobes such as acetate producers, methanogens, and sulfate reducers in a P-impacted site than a pristine site of the Florida Everglades. It is likely that there is an abundant supply of electron acceptors that these anaerobes can utilize. The surface water and soil pore water in WCA-2A had relatively high SO_4^{-2} concentrations due to groundwater seepage (Schipper and Reddy, 1994). Thus, SO_4^{-2} may be non-limiting to sulfate reducers, explaining the correlation between microbial biomass and SIPM under sulfate-reducing conditions. Under HCO_3-reducing conditions, the considerable number of sulfate reducers outcompete the methanogens for available substrate, hence reducing the population of the latter. Therefore, any conversion of HCO_3^- to CH_4 that is coupled with P_o mineralization is inhibited.

Except under NO_3-reducing conditions, labile P_o was positively correlated ($P<0.05$) with SIPM under all reducing conditions. It is likely that P_o mineralization under NO_3-reducing conditions in detritus and 0-10 cm soils with high amounts of labile P_o conforms to first-order rather than zero-order kinetics.

Microbial Biomass P

Microbial biomass P decreased with soil depth. There was no significant difference in microbial biomass P with type of electron acceptor regardless of depth (Table 3.3). It appears that O_2 availability or redox status has no effect on microbial biomass P, while soil depth affects the number or population of microorganisms that would be present. Water table drawdown does not affect microbial respiration in peatlands (Freeman et al., 1996). It is probable that water moving into the micropore spaces where most bacteria thrive does not easily and completely fill up these spaces; hence, bacterial growth is not prevented. Effective aeration of a wetland can increase microbial activity, and aerobic metabolism is more efficient than anaerobic metabolism (Stanier et. al., 1977).

Table 3.3. Microbial biomass P of detritus and soils incubated with various electron acceptors for 15 d at 30°C.

Depth	HCO_3^-	SO_4^{-2}	NO_3^-	O_2
	----------mg Pkg^{-1}----------			
detritus	173 (57)a	206 (54)a	208 (49)a	182 (74)a
0-10	73 (19)b	78 (20)b	79 (19)b	70 (15)b
10-30	23 (4)c	23 (11)c	19 (10)c	19 (13)c

Values in parentheses are standard deviations; Values with the same letters within a row or column are not significantly different at $\alpha = 0.05$.

Effect of N Addition on SIPM and PMP

This experiment was conducted to determine whether additional NO_3-N amendment is required to maintain non-limiting conditions for zero-order kinetics. There was a considerable amount of NO_3-N (0.8 mg/bottle) consumed after the 15-d incubation of detritus and 0-10 cm soil (data not shown). This leads us to speculate that zero-order kinetics may not be appropriate to describe rates of P_o mineralization. There was no significant difference in SIPM under two levels of NO_3-N addition for both detritus and 0-10 cm soil (Table 3.4). Likewise, the PMP with 0.8-mg NO_3-N was not different from PMP with 1.6-mg NO_3-N in both detritus and 0-10 cm soil, suggesting that NO_3^- was not a limiting factor in P_o mineralization.

Table 3.4. Effect of nitrate addition on potentially mineralizable-P (PMP) and substrate-induced P mineralization (SIPM).

Depth	NO_3-N added	PMP	SIPM
	mg	mg $kg^{-1}d^{-1}$	mg $kg^{-1}h^{-1}$
detritus	0.8	9.12 (5.66)a	30 (10)a
	1.6	9.54 (7.58)a	25 (15)a
0-10	0.8	1.47 (1.94)b	14 (8)b
	1.6	2.72 (1.68)b	8 (5)b

Means are the averages for four stations and values in parentheses are standard deviations. Means with the same letter within a column are not significantly different at $P<0.05$.
PMP and SIPM were calculated from the net change of combined $NaHCO_3$- and HCl-P_i.

Potentially mineralizable-P decreased with soil depth and is associated with the higher microbial biomass P, labile P_o, and P_i pools in detritus than in soils. The influence of P enrichment on PMP was not apparent for the detrital layer, where labile P_o substrates are non-limiting. Net mineralization was also less distinct in detritus with P_i being immediately immobilized after P_o mineralization under aerobic condition. For the 0-10 cm soil, P enrichment affected P_o mineralization, with higher rates for the impacted and transitional than non-transitional stations. Aeration, however, did not affect labile P_o substrates present at the 0-10 cm depth. For the 10-30 cm soils, low amounts of labile P_o limit P_o mineralization.

Similar to PMP, SIPM decreased with soil depth. There was no effect of electron acceptors on SIPM in detritus that has less limiting labile P_o substrates. The conclusion that aerobic metabolism being more efficient than anaerobic metabolism was verified during short-term incubations of 0-10 and 10-30 cm soils. Substrate-induced P mineralization in the 0-10 cm depth was higher under aerobic compared to anaerobic conditions, and decreased in the order: $O_2 > NO_3^- > SO_4^{-2} = HCO_3^-$ in the 10-30 cm depth.

With microbial biomass and APA exhibiting differences in governing P_o mineralization may suggests that alkaline phosphatases were from sources other than microorganisms or electron acceptors were non-limiting. The influence of mass C:P_o ratio on PMP similarly was not apparent in the presence of electron acceptors.

Microbial biomass yielded positive correlations with SIPM under O_2- and SO_4^{-2}-reducing conditions but not under NO_3^- - and HCO_3^--reducing conditions. Competition against sulfate-reducers for substrates by methanogens limits P_o mineralization under HCO_3^--reducing conditions. The comparable probable numbers of aerobes and anaerobes

in WCA-2A (Drake et al., 1996) can explain why the availability of oxygen does not affect the microbial biomass pool. This study, therefore, suggests that the effects of various electron acceptors have to be investigated together with other factors such as the nature and amount of labile P_o.

Conclusions

The electron acceptors (O_2, NO_3^-, SO_4^{-2}, HCO_3^-) affected P_o mineralization during short-term incubation of soils that had limiting labile P_o substrates. Substrate-induced P mineralization for soils revealed higher P_o mineralization rates under aerobic than anaerobic conditions, while SIPM for detritus proceeded at similar rates with variable electron acceptors. With 15-d incubation, the type of electron acceptor did not affect P_o mineralization in soils, while the P_i released through mineralization of detritus is immobilized under O_2-reducing condition. The study demonstrated that the microbial population does not respond to varying electron acceptor during long incubation such as 15 d. However, the microbial population exhibited increases in metabolic activities during short-term dry periods.

This study also demonstrated that in Ca-enriched ecosystems, P_o mineralization involving long-term incubation should account for precipitation of P_i released through mineralization. Precipitation, however, becomes negligible during short-term incubation periods such as 4 h.

In wetlands subject to wet and dry cycles, short-term aerobic condition can increase mineralization of labile P_o. However, longer drying of wetlands allows for precipitation or adsorption of P_i released after mineralization. Further study is needed to

determine the maximum time a wetland can be kept or maintained dry while minimizing the release of P_i due to P_o mineralization. The information that can be obtained from such study will be beneficial in wetlands maintaining to function as P sinks while experiencing intermittent hydrological fluctuations.

CHAPTER 4
INFLUENCE OF PHOSPHORUS-HYDROLYZING ENZYMES AND SUBSTRATE QUALITY ON ORGANIC PHOSPHORUS MINERALIZATION IN FRESHWATER WETLANDS

Introduction

The breakdown of complex polymers into simple monomers or oligomers by extracellular enzymes can be the rate-limiting step in the microbial decomposition of organic matter (Sinsabaugh et al., 1993). Hydrolysis of organic matter through enzyme activity can result in 60-70% loss of detrital mass (Harrison and Mann, 1975). Extracellular enzymes excreted into the soil as free enzymes or cell-bound (Burns, 1982) mediate the release of P bound to organic matter.

Phosphatases that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Feder, 1973) are classified into phosphomonoesterase, phosphodiesterase, triphosphoric monoester hydrolases, hydrolases acting on phosphoryl-containing anhydrides, and phosphoamidases or other enzymes acting on P-N bonds (Eivazi and Tabatabai, 1977). The synthesis or activation of these enzymes is repressed by P enrichment or derepressed in response to P depletion (Speir and Ross, 1978; Spiers and McGill, 1979; Newman et al., 2000; Kang and Freeman, 1999). An inverse relationship between phosphatase activity and inorganic P (P_i) concentration has been reported for wetlands and aquatic systems (Newman and Reddy, 1993; McLatchey and Reddy, 1998; Kang and Freeman, 1999).

The type of substrate also has an indirect effect on P_o mineralization. Bacterial synthesis of phosphatase depends on the type of organic P (P_o) substrate available (Chróst, 1991). Little is known about the decomposition of much of the identified soil P_o. Knowledge of the mineralization of individual P_o constituents in soil can provide a better prediction of the rate of P_o mineralization.

The quality of substrate for microbial metabolism can influence nutrient cycling in wetlands. Plants that grow in nutrient-poor ecosystems yield poor-quality litter that decomposes slowly, while those grown in nutrient-rich ecosystems produce readily degradable litter (Hobbie, 1992). Plants are sources of enzymes and, at the same time, contribute to favorable conditions for microbial synthesis of enzymes (Gianfreda and Bollag, 1996). Plant-derived polymers are broken into simple monomers through the action of extracellular enzymes (Benner et al., 1984). The decomposition of plants can affect the microbial community by shifting the bacterial population from aerobic to anaerobic microorganisms (Kistritz, 1978); therefore, decomposing biomass is important to the nutrient cycling of wetlands.

Eutrophication of pristine wetlands raises questions concerning the role of remineralization of labile organic P as well as the influence of the nature of P_o substrate, in addition to microbial and enzyme activities. Several studies have indicated that phosphatases decompose significant fractions of soluble organic P in lakes and marine environments (Berman, 1970; Herbes et al., 1974; Kobori and Taga, 1979a; Newman and Reddy, 1993). In wetlands, the significance of phosphatases as well as the effects of the nature of P_o on P mineralization, however, is less studied. Alkaline phosphatase activity

measured in periphyton (Newman et al., 2000) and soils (Wright and Reddy, 2000) is regarded as a sensitive indicator of P eutrophication in freshwater wetlands.

Thus, the objectives of the study were to: (i) determine the effect of P enrichment on the phosphomono- and phophodiesterase activities of freshwater wetlands, (ii) determine the influence of P enrichment on phosphatase hyrolyzable-P, (iii) examine the influences of selected P pools on phosphomono- and phosphodiesterase activities, (iv) determine the influence of the nature of P_o substrate and P enrichment on P mineralization in freshwater wetlands, and (v) determine the influence of added plant residues on mineralization of labile organic P.

Materials and Methods

Detritus and Soil Sampling

Water Conservation Area - 2A:

Soils and detritus/floc were collected at selected locations along two sampling transects in WCA-2A (Fig. 4.1). Along F-transect, detritus/floc and soils were collected at stations U3 (10.8 km from the S10C water inflow) and F1 (1.84 km from the S10C water inflow) on May 1998. Along W-transect, detritus and 0-10 cm soil were obtained from three selected stations on March 1999. The stations were located at 2.3, 5.1, and 10.1 km from the water inflow and represented impacted, transitional, and non-impacted stations, respectively. Detritus consisted of recognizable plant materials such as cattail or sawgrass that are loosely associated and on the surface of a more compact peat soil. The detrital layer varied in thickness from less than 1 cm for the sawgrass areas to >25 cm in

the cattail areas. Floc in the non-impacted stations consisted of sawgrass, with remaining benthic calcareous periphyton.

For the F-transect, detritus/floc was removed from the surface and stored in polyethylene bags. Using an aluminum pipe, three cores were obtained from each station. Pipe was driven to a depth of 30 cm below the surface. Cores that had less than 5% compaction were capped and removed from the ground. Each core was extruded and sectioned into 0-5, 5-10, 10-20, and 20-30 cm depth increments. Each section was transferred to a polyethylene bag. The bags were stored in an ice chest while transported to the laboratory for storage at 4°C. In the laboratory, bulk samples were homogenized by removing live and large roots. Water content was determined by drying a known weight of wet subsample at 70°C to constant weight.

For the W-transect, soil cover was obtained as described previously. Soil from the 0-10 cm depth was bagged and the remainder of the core was returned to the ground. Samples were kept on ice and transported to the laboratory for storage at 4°C. In the laboratory, bulk weights and moisture contents were determined as described previously.

Blue Cypress Marsh:

Blue Cypress Marsh (BCM) is located in the upper St. Johns River Basin. Two stations in the marsh were identified as Northeast impacted (NEI) and unimpacted Central (UIC) based on vegetation (Fig. 4.2). The impacted and non-impacted stations consisted predominately of cattail and sawgrass, respectively. At each station, four cores located approximately 1m apart were collected using a coring tube that was driven 50 cm below the surface. Each core was immediately extruded into 0-10, 10-20, 20-30 and 30-50 cm depth increments. Soil samples were passed through a 0.5 cm^2 sieve to remove

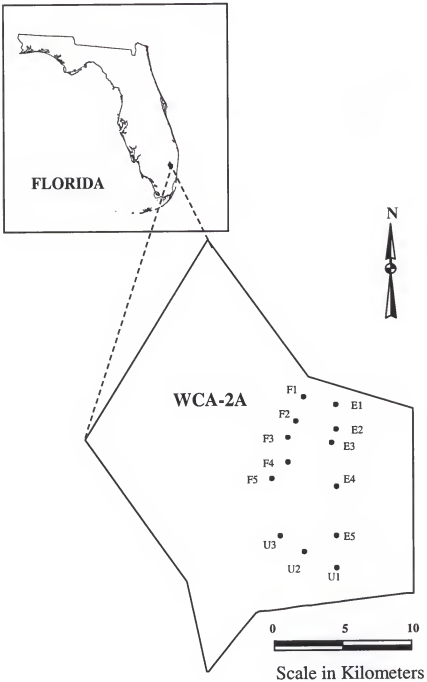

Fig. 4.1. A map of Water Conservation Area -2A showing the F and U transects.

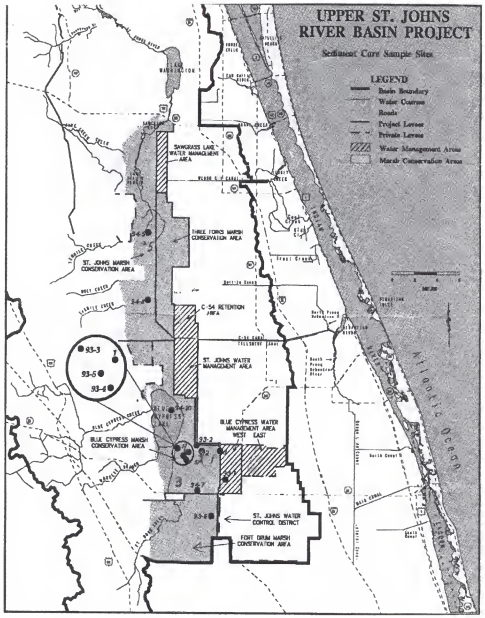

Fig. 4.2. Map of Upper St. Johns River Basin showing the Blue Cypress Marsh as encircled.

large roots and debris before homogenization. Samples were stored at 4°C until analysis.

Sample Handling and Preparation

The 0-10 cm soils from the W-transect were mixed with either sawgrass or cattail residues as follows: Standing dead sawgrass and cattail leaves were obtained from stations U3 and E2 located 10.8 and 2.25 km, respectively from the S10-C inflow structure in WCA-2A. The plant materials were dried at 70°C, and then ground and passed through a 20-mesh sieve.

At each station, 150 g of the soil from each of the three cores were combined and mixed. About 1.5 g of ground plant material was mixed with 150 g of the composited fresh soil using 80 mL distilled H_2O. A 10-g subsample of the soil-plant mixture was dried to constant weight at 70°C.

Bottles (triplicates) were designated for destructive analysis of substrate-induced P mineralization (SIPM), microbial biomass P, and phosphomonoesterase (alkaline phosphatase) and phosphodiesterase activities. Five grams of the mixed soil-plant sample were weighed into glass serum bottles in two sets. Each bottle was amended with 2 mL of deionized distilled water before being capped and sealed with a rubber septum and aluminum. The bottles were evacuated for 10 s using 100 kPa suction and purged with N_2 gas for 3-4 min. The first set was analyzed immediately, while the second set was incubated for 30 d at 30°C.

Microbial Biomass P

Samples from each core and station were analyzed for microbial biomass P as follows: Triplicate 5-g subsamples were weighed into 50-mL polyethylene centrifuge tubes and equilibrated with 25 mL 0.5 M $NaHCO_3$ (pH = 8.5) for 16 h with end-to-end

shaking at low speed. The sample was centrifuged at 4066 × *g* for 10 min before the supernatant was passed through a 0.45 µm membrane filter. Another 5-g subsample was weighed into 50-mL centrifuge tubes, in triplicate. Each tube was amended with 1 mL of $CHCl_3$ and capped for 24 h. After equilibration with $CHCl_3$, the tubes were uncapped and set in a fume hood for another 24 h. When $CHCl_3$ had dissipated, samples were equilibrated with 0.5 M $NaHCO_3$ and filtered as described previously.

A 5-mL aliquot of the extracts was analyzed for total P (TP) using a $K_2S_2O_8$-H_2SO_4 digestion (Bowman, 1989), followed by an automated SRP analysis (USEPA, 1993). Microbial biomass P in mg kg^{-1} was calculated as the difference in TP between the $CHCl_3$-treated and control samples and reported uncorrected for efficiency. Microbial biomass P also was determined for mixed soil-plant samples incubated for 30 d.

Phosphorus-hydrolyzing Enzymes

Alkaline or acid phosphatase and phosphodiesterase activities were determined for homogenized soils and detritus using the procedure of Tabatabai and Bremner (1969) and Browman and Tabatabai (1978), respectively. The alkaline nature of soils and detritus from WCA-2A necessitated measurement of alkaline phosphatase activity (APA), while acid phosphatase activity was measured for soils from BCM having pH between 5 and 6. Alkaline and acid phosphatase activities were measured using modified universal buffer (MUB) at pH 11.0 and 6.5, respectively. For the mixed soil-plant samples, alkaline phosphatase and phosphodiesterase activities were determined before and after the 30-d incubation.

Alkaline/Acid Phosphatase Activity:

For both acid and alkaline phosphatases, 0.5-g dry equivalent of fresh sample was weighed into polyethylene centrifuge tube in duplicate. The first tube was amended with 250 μL of toluene, 1 mL of *p*-nitrophenyl phosphate (0.032 M), and 4 mL of MUB. The contents of each tube were blended with a vortex mixer and then incubated at 37°C for 1 h. After incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH was added to each tube. The tube then was shaken and the soil suspension was passed through a Whatman no. 2 filter paper. *Para*-nitrophenol in the filtrate was measured at 410 nm using an ultraviolet-visible spectrophotometer (UV-160, Shimadzu Corp, Japan).

To the remaining (control) tube, 250 μL of toluene and 4 mL of MUB were added and mixed. One mL of *p*-nitrophenyl phosphate was added to this mixture before it was passed through Whatman no. 2 filter paper. *Para*-nitrophenol was measured as described previously.

Standards of 0, 5, 10, 20, 30, 40, and 50 μg *p*-nitrophenol were prepared as follows: Volumes of 0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL of the 10 μg mL^{-1} solution were delivered into 20-mL glass vials. Distilled water was added to produce 5 mL total volume. One mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to each vial and mixed. The mixture was filtered and *p*-nitrophenol was measured as described previously.

Phosphodiesterase Activity:

Approximately 0.5-g dry equivalent of fresh sample was weighed into 50-mL polyethylene centrifuge tube in duplicate. Two hundred fifty μL of toluene, 1 mL of

0.005 M bis *p*-nitrophenyl phosphate as substrate, and 4 mL of 0.05 M tris-hydroxymethyl amino methane (THAM; pH = 8) were added to the first tube, and the mixture was incubated at 37°C for 1 h. After incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M THAM (pH = 12) as extracting solution were added to each tube. The mixture was passed through Whatman no. 2 filter and *p*-nitrophenol released in the filtrate was measured at 410 nm using an ultraviolet-visible spectrophotometer.

To the second tube, 250 µL of toluene and 4 mL THAM (pH=8.0) were added and mixed. One mL of 0.005 M bis *p*-nitrophenyl phosphate was added immediately before the mixture was passed through Whatman no. 2 filter. The amount of *p*-nitrophenol released was calculated from the calibration curve using the same range of standard *p*-nitrophenol as used for the alkaline/acid phosphatase assay.

Potentially mineralizable-P (PMP) and Substrate-induced P Mineralization

Potentially mineralizable-P was determined on homogenized detritus and soil using the procedure described in Chapter 2. Potentially mineralizable-P with and without accounting for precipitation of P_i released from organic P were calculated as described in Chapter 2.

The mixed soil-plant samples were analyzed for SIPM before and after incubation at 30°C for 30 d using the procedure described in Chapters 2 and 3.

Phosphatase-hydrolyzable P

The amount of native P_o hydrolyzable by alkaline phosphatase was determined for detritus and soils obtained from three select stations along the W transect. Detritus or soil was homogenized in a food processor before a 10-g subsample was dried to constant weight at 70°C. One gram of fresh sample as a control was weighed into 50-mL

centrifuge tubes in triplicate. Another 1-g fresh sample as treated was weighed into 50-mL centrifuge tubes in triplicate. The tubes were capped loosely and autoclaved at 121°C for 20 min. When cooled, each tube received 2 mL of previously sterilized modified universal buffer (MUB) (pH = 7.0±0.5).

Alkaline phosphatase solution was freshly prepared in sterilized MUB as follows: 0.5 mL of stock alkaline phosphatase having 216 enzyme units (3.6 mg protein mL^{-1}; 60 units mg^{-1} protein) was pipeted into a sterilized 25-mL volumetric flask and brought to volume with sterilized MUB. A half mL of alkaline phosphatase solution was added to each tube designated as treated. The tubes were capped immediately and contents mixed before being incubated at 30°C for 4 h. After incubation, samples were equilibrated with 5 mL of 0.5 M $NaHCO_3$ (pH = 8.5) with end-to-end shaking for 30 min. The sample suspension was centrifuged at 4066 × *g* for 10 min and the supernatant was passed through a 0.45 µm membrane filter (Gelman Sciences, Ann Arbor, MI).

For the control tubes, 0.5 mL of sterilized MUB was added before equilibration and filtered as described previously for the incubated samples. Filtrates were analyzed for SRP by the Murphy and Riley method (1962) using an ultraviolet-visible spectrophotometer.

Phosphatase hydrolyzable-P was calculated as:

$$\{(NaHCO_3\text{-}P_i)_{incubated} - (NaHCO_3\text{-}P_i)_{control}\}/t$$

where $(NaHCO_3\text{-}P_i)_{incubated}$ is SRP extracted by $NaHCO_3$ in samples incubated with alkaline phosphatase for 4 h in mg kg^{-1}; $(NaHCO_3\text{-}P_i)_{control}$ is SRP extracted by $NaHCO_3$ in samples without alkaline phosphatase in mg kg^{-1}; and t is incubation time. Phosphatase hydrolyzable-P was reported as mg kg^{-1} h^{-1}.

Mineralization of Selected Organic P Substrates

The influence of P enrichment on the mineralization of added organic P was determined over a 15-d incubation for 0-10 cm soils from U3 (10.8 km from the inflow point) and E2 (3.3 km from the inflow point) of WCA- 2A. Three cores taken at each station were combined and mixed thoroughly to represent impacted (E2) and non-impacted (U3) soils.

To determine the effect of aeration, mineralization of various P_o substrates was investigated for both fresh and drained soils. Composite sample was homogenized before a portion was spread thinly onto a 20-cm x 28-cm type A/E glass filter (Gelman Sciences, Ann Arbor, MI) and allowed to drain for 30 min. Moisture content of drained soil was determined in the same fashion as described previously.

Half-gram dry equivalent of drained soil was weighed and spread thinly on a medium-size hexagonal plastic weighing dish in five sets of triplicates. Four sets were spiked with the organic P substrate while a set received deionized distilled water. Each organic P (P_o) substrate - glucose-6-phosphate, glycerophosphate, phytic acid, or adenosine triphosphate (ATP) (Sigma Chemical Co., St. Louis, MO) - was prepared as 200 mg P L^{-1} in deionized distilled water. Five hundred µL of each P_o solution was spread uniformly into the corresponding dish while 500 µL of distilled water was spread on each dish labelled as a control. The dishes were randomly arranged in a 20-cm × 28-cm rack that was later covered with polyethylene film to protect samples from condensate. The rack was set in a 46-cm high plastic container filled with distilled water to about the 20-cm depth. Water was added in the container to prevent the samples from

drying during incubation. To allow air exchange during incubation, the container was loosely covered before being incubated at 30°C.

Five sets of homogenized fresh samples were weighed into 60-mL serum bottles in triplicate. One-mL of distilled water was added to each bottle before it was capped with a butyl rubber stopper and sealed with aluminum crimps to create reducing conditions. The bottles were evacuated for 10 s using a 100-kPa suction and purged with O_2-free N_2 gas for 3-4 min. The sets were divided into a control and four P_o substrates. Five hundred μL of each P_o solution, previously bubbled with N_2 gas for 4 min, was added into corresponding serum bottle. Five hundred μL of deionized distilled water previously bubbled with N_2 gas was added to each control serum bottle. The serum bottles were incubated at 30°C.

To allow for destructive sampling, weighing dishes and serum bottles were prepared for incubation at 4, 24, 48, 96, 168, and 360 h. At the end of incubation, 25 mL of 0.5 M $NaHCO_3$ (pH = 8.5) was added to each bottle while the sample from dish was transferred quantitatively into a 50-mL polyethylene centrifuge tube using the same extractant. The soil suspension was equilibrated for 30 min, and centrifuged at 4066 × *g* for 10 min. The supernatant was passed through a 0.45-μm membrane filter (Gelman Sciences, Ann Arbor, MI).

For fresh and drained samples incubated at 4 h and 7 d, the residue that remained after extraction with 0.5 M $NaHCO_3$ was equilibrated with 25 mL of 1 M HCl for 3 h. After equilibration, the soil suspension was centrifuged and filtered as described previously.

Analytical Methods

Soluble reactive P in 0.5 M $NaHCO_3$ extracts was analyzed by the ascorbic acid method (Murphy and Riley, 1962) using an ultraviolet-visible spectrophotometer. Soluble reactive P in HCl filtrates, on the other hand, was determined by the ascorbic acid method using an autoanalyzer (USEPA, 1993).

Total C and N contents were determined using a Carlo-Erba NA-1500 CNS analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P was determined by HNO_3-$HClO_4$ digestion (Sommers and Nelson, 1972), followed by the ascorbic acid method using an autoanalyzer (USEPA, 1993).

Statistical Analyses

Comparison between mean values for the parameters – $NaHCO_3$-P_i, HCl-P_i, microbial biomass P, total labile P_o, total P, and phosphatase activities - for both sites was made using a two-tailed t-test. Difference among mean values was determined using Fisher's least significant difference (LSD) in SAS version 6.12. For each depth, phosphatase hydrolyzable-P was differentiated among stations using PROC GLM in SAS version 6.12, and means were separated using least significant difference (LSD). Relationships between potentially mineralizable-P (PMP) and various parameters were derived from linear regression analysis using Microsoft Excel Version 7.0.

Results

Soil and Detritus Characterization

In WCA-2A, soil bulk density and C:N mass ratio generally increased with depth (Table 4.1). The pH of the 0-10 cm soils and detritus was above neutral (Table 4.2).

Table 4.1. Selected physico-chemical characteristics of floc and soils in WCA-2A.

Depth	Bulk Density	C:N	Total P	$NaHCO_3$-P_i	HCl-P_i	Microbial P	Labile P_o
	g cm^{-3}			----------mg kg^{-1}----------			
F1[†]							
floc	n. d.	12.2 ±0.6	1960 ±78	21.9 ±7.0	418 ±88	359 ±87	464 ±99
0-5	0.05 ±0.00	14.2 ±0.5	1657 ±24	13.8 ±3.9	431 ±192	188 ±57	256 ±77
5-10	0.07 ±0.01	16.1 ±2.1	1592 ±229	5.5 ±0.2	371 ±110	55 ±13	80 ±12
10-20	0.08 ±0.01	17.4 ±2.7	1293 ±90	9.3 ±0.6	486 ±168	64 ±3	104 ±3
20-30	0.10 ±0.02	16.2 ±0.9	1139 ±24	8.6 ±3.2	245 ±152	52 ±13	79 ±21
Mean±SD	0.07 ±0.02	15.2 ±2.0	1528 ±322	11.8 ±6.4	390 ±91	144 ±133	197 ±166
U3[‡]							
floc	n. d.	10.7 ±0.2	574 ±146	0.0 ±0.0	137 ±9	161 ±20	172 ±18
0-5	0.07 ±0.02	13.0 ±1.3	563 ±69	0.9 ±1.5	146 ±68	94 ±30	106 ±24
5-10	0.07 ±0.00	13.7 ±0.7	482 ±58	3.3 ±3.4	58 ±68	34 ±17	46 ±16
10-20	0.07 ±0.01	12.7 ±0.4	267 ±64	3.6 ±0.6	15 ±2	36 ±6	46 ±8
20-30	0.10 ±0.02	13.8 ±0.8	162 ±47	2.0 ±0.6	11 ±2	22 ±10	30 ±13
Mean±SD	0.08 ±0.01	12.8 ±1.3	410 ±185	1.9 ±1.5	73 ±65	69 ±59	80 ±59

n.d. = not determined

[†] 1.84 km from the water inflow point.

[‡] 10.8 km from the water inflow point

Values are means ± standard deviation among 3 cores.

Table 4.2. Selected physico-chemical characteristics of soils collected from Blue Cypress Marsh in December 1998.

Depth	Bulk density	pH	% Ash	C/N	Total P	$NaHCO_3$-P_i	HCl-P_i	Microbial P[†]	Labile P_o[‡]
	$g\ cm^{-3}$					mg kg^{-1}			
Unimpacted Central (UIC)									
0-10	0.07	5.8 ±0.1	7.0 ±0.2	16.6 ±1.3	530 ±16	22.7 ±3.5	9.6 ±2.1	95 ±21	111 ±19
10-20	0.06	5.9 ±0.1	6.9 ±0.4	14.9 ±0.1	437 ±18	9.9 ±1.7	5.6 ±0.7	80 ±9	97 ±8
20-30	0.07	5.9 ±0.1	6.7 ±0.3	15.8 ±0.8	368 ±20	7.8 ±2.2	3.2 ±1.2	66 ±5	83 ±6
30-50	0.07	5.9 ±0.1	6.9 ±1.8	16.7 ±0.9	280 ±29	5.4 ±0.7	3.0 ±1.1	45 ±7	59 ±9
Mean± SD	0.07	5.9 ±0.1	6.9 ±0.1	16.0 ±0.8	404 ±106	11.4 ±7.8	5.3 ±3.0	71 ±21	87 ±22
Northeast Impacted (NEI)									
0-10	0.06	5.8 ±0.1	7.4 ±0.5	14.3 ±0.9	754 ±75	28.2 ±10.7	18.2 ±5.5	76 ±18	99 ±14
10-20	0.08	5.8 ±0.1	6.5 ±0.1	14.1 ±0.3	320 ±36	18.1 ±1.2	7.7 ±1.1	40 ±12	55 ±9
20-30	0.06	5.9 ±0.0	5.5 ±0.1	14.3 ±0.3	258 ±12	17.3 ±1.9	0.4 ±0.6	55 ±18	68 ±19
30-50	0.07	6.0 ±0.0	6.6 ±0.2	15.0 ±0.4	191 ±14	10.6 ±1.9	3.6 ±1.1	29 ±6	38 ±5
Mean± SD	0.07	5.9 ±0.1	6.5 ±0.8	14.4 ±0.4	381 ±254	18.5 ±7.2	7.5 ±7.8	50 ±21	65 ±26

Values are averages ± standard deviation among 4 cores.

[†] difference between TP of fumigated and TP of non-fumigated samples, and reported uncorrected for efficiency.

[‡] difference between TP of fumigated and SRP of non-fumigated samples.

Meanwhile, total P, microbial biomass P, and labile P_o generally decreased with soil depth. Microbial biomass P ($P<0.10$; n=15) was higher for the P-impacted than non-impacted stations. However, microbial biomass as a fraction of TP in the non-impacted area was higher ($P<0.01$; n=15) than for the impacted stations. Similar to microbial biomass P, the P-impacted stations had higher total labile P_o ($P<0.01$; n=15) than the non-impacted stations. Total P of detritus ($P<0.001$; n=6) and soils ($P<0.001$; n=15) were higher at the impacted than non-impacted stations. Higher TP near the inflow than in the interior of the marsh resulted to a lower C:P mass ratio. The inorganic pools ($NaHCO_3$-P_i and HCl-P_i) for station F1 and HCl-P_i for station U3 decreased with soil depth (Table 4.3).

In contrast to soils and detritus of WCA-2A, soils in BCM had slightly acid pH between 5.8 and 6.0 (Table 4.3). There was no trend in C:N ratio with depth, but the range of C:N ratios was comparable to WCA-2A. The ash contents of soils in BCM (5.5-7.4%) were lower than values reported for WCA-2A (DeBusk et al., 1994). In BCM, TP generally decreased with soil depth while average TP between NEI and UIC was not significantly different. Total P in the impacted station (NEI), however, was higher ($P<0.05$; n=16) than in the non-impacted station (UIC) for the 0-10-cm depth. Microbial biomass as a fraction of TP was not different between the stations and was 16-18% and 10-20% of TP at UIC and NEI, respectively. Total labile P_o pool, on the other hand, was higher ($P<0.05$; n=16) at UIC than NEI. Both microbial biomass P and labile P_o pools, however, decreased with soil depth in both UIC and NEI.

Total P in cattail residues was 365 mg kg^{-1} while it was 32 mg kg^{-1} in sawgrass residues. Total C contents of cattail and sawgrass residues were 458 and 449 g kg^{-1},

Table 4.3. Selected physico-chemical characteristics of detritus and soils collected from selected stations along W transect in WCA-2A on March 1999 and from stations E2 and U3 on July 1998.

Station	Distance from inflow	pH	Bulk density	C:N	Total P	$NaHCO_3$-P_i	HCl-P_i	Microbial P[†]	Labile P_o[‡]
	km		g cm^{-3}		----------	mg kg^{-1}	----------		
detritus									
W2	2.3	7.35 ±0.03	n. d.	15.5 ±0.7	1893 ±36	14.8 ±1.9	206 ±5	363 ±115	515 ±121
W5	5.1	7.37 ±0.07	n. d.	16.2 ±1.7	1051 ±55	0.2 ±0.2	125 ±29	288 ±5	326 ±10
W8	10.1	7.73 ±0.08	n. d.	12.4 ±1.6	693 ±267	0.0 ±0.0	139 ±31	151 ±74	172 ±69
0-10 cm soil									
W2	2.3	7.40 ±0.08	0.09 ±0.01	15.2 ±0.5	796 ±287	2.9 ±0.1	124 ±83	43 ±11	78 ±16
W5	5.1	7.35 ±0.04	0.10 ±0.02	16.5 ±3.0	688 ±173	2.0 ±1.2	83 ±29	107 ±43	135 ±48
W8	10.1	7.54 ±0.11	0.09 ±0.00	13.0 ±0.6	336 ±80	0.4 ±0.4	34 ±20	46 ±11	66 ±11
E2	3.3	n. d.	n. d.	13.8	992 ±20	10.0 ±0.7	112 ±7	83	125
U3	10.8	n. d.	n. d.	11.5	277 ±9	2.8 ±0.1	29 ±2	18	57

n.d. = not determined; Values are means ± standard deviation for three cores.

[†] difference between TP of fumigated and TP of non-fumigated samples, and reported uncorrected for efficiency.

[‡] difference between TP of fumigated and SRP of non-fumigated samples.

respectively. The resulting mass C:P ratios were 1255 for cattail and 14031 for sawgrass. Total N content of cattail was threefold higher than for sawgrass. These differences in chemical composition between sawgrass and cattail may indicate difference in turnover along with structural attributes e.g., more lignin in sawgrass (Day, 1982; Davis, 1991; DeBusk and Reddy, 1998).

Phosphatase and Phosphodiesterase Activities

Phosphatase assay was conducted at pH 6.5 for BCM soils and at pH 11.0 for soils and detritus of WCA-2A to obtain optimum enzyme activity (Eivazi and Tabatabai, 1977). The activities of both phosphatase and phosphodiesterase were determined to evaluate which of these P-hydrolyzing enzymes control P_o mineralization in BCM and WCA-2A.

Acid phosphatase activity decreased with soil depth (Table 4.4). However, there was no difference in acid phosphatase activity between UIC and NEI. Unlike BCM, there was a significant difference in APA between the non-impacted (U3 and W8) and impacted (F1 and W2) stations of WCA-2A. The non-impacted stations had higher ($P<0.05$; n=15) APA than the impacted stations. Likewise, phophodiesterase activity in the non-impacted stations was higher ($P<0.10$; n=15) than for impacted stations of WCA-2A. In contrast, phosphodiesterase activity in BCM was higher ($P<0.01$; n=16) at the impacted (NEI) than non-impacted (UIC) stations.

There was no significant relationship obtained when acid phosphatase activity was plotted as function of phosphodiesterase activity (Fig. 4.3). In contrast, there was a significant relationship ($r^2 = 0.80$) obtained between APA and phosphodiesterase activities

Table 4.4. Phosphatase activities at sampling stations in Blue Cypress Marsh (BCM) and Water Conservation Area-2A (WCA-2A).

Sampling date	Station	Distance from inflow	Depth	Phosphatase activity	Phosphodiesterase activity
		km	cm	mg *p*-nitrophenol kg^{-1} h^{-1}	
BCM					
Dec. 1998	UIC[†]	n.d.	0-10	1745 ±392	37 ±9
			10-20	1505 ±410	31 ±11
			20-30	1264 ±283	30 ±1
			30-50	1388 ±228	19 ±6
	NEI[‡]	n.d.	0-10	1631 ±171	67 ±20
			10-20	1658 ±225	32 ±8
			20-30	840 ±164	43 ±12
			30-50	819 ±104	42 ±6
WCA-2A					
May 1998	U3	10.8	floc	1788 ±1219	1292
			0-5	2219 ±283	399 ±109
			5-10	361 ±130	44 ±3
			10-20	474 ±208	29 ±7
			20-30	207 ±191	23 ±20
	F1	1.84	floc	386 ±128	96 ±27
			0-5	643 ±87	111 ±40
			5-10	243 ±118	36 ±7
			10-20	341 ±116	42 ±11
			20-30	252 ±94	29 ±12
Mar. 1999	W2	2.3	detritus	305 ±20	156 ±29
	W5	5.1		1579 ±246	457 ±188
	W8	10.1		2109 ±805	2052 ±1256
	W2	2.3	0-10	89 ±30	23 ±2
	W5	5.1		608 ±197	164 ±55
	W8	10.1		693 ±355	134 ±69

n.d. = not determined; values are means ± standard deviation among 4 cores for Blue Cypress Marsh and 3 cores for Water Conservation Area-2A.

[†] Unimpacted Central

[‡] Northeast Impacted

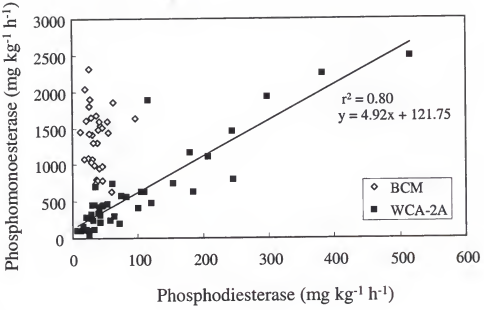

Fig. 4.3. Phosphomonoesterase activity as a function of phosphodiesterase activity in soils. Phosphomonoesterase activities were measured at pH =11.0 for WCA-2A and at pH = 6.5 for BCM.

for WCA-2A. The slope indicated that APA was five times greater than phosphodiesterase activity.

Potentially mineralizable-P (PMP)

In WCA-2A, the detritus/floc had higher PMP than the 0-10 cm soil increment (Table 4.5). Alkaline phosphatase activity in detritus or floc was negatively correlated with PMP though it was not correlated with PMP in soils. In both BCM and WCA-2A, the PMP rates did not correlate with phosphodiesterase activity. Similarly, the microbial biomass and total labile P_o did not correlate with PMP for both detritus and soils of WCA-2A. In BCM, these P pools correlated with PMP at $P<0.05$.

Phosphatase hydrolyzable-P_o

Native P_o that releases P_i after hydrolysis by phosphatase is considered easily hydrolyzable (Kobori and Taga, 1979a). To obtain the potential amounts of easily hydrolyzable P_o, enzyme was added in non-limiting concentrations as determined in preliminary experiments. Under non-limiting enzyme concentrations, both detritus and soils demonstrated net mineralization for all stations under different P enrichment (Fig. 4.4). The detritus had higher ($P<0.01$) phosphatase hydrolyzable-P than did the 0-10 cm soils. For detritus, phosphatase hydrolyzable-P was different ($P<0.01$) among stations and decreased in the order: impacted > transitional > non-impacted. For the 0-10 cm soil, phosphatase hydrolyzable-P_o in impacted and non-impacted areas was not significantly different. Hydrolyzable-P_o in the transitional area, however, was higher than for the non-impacted area. There was no difference in phosphatase hydrolyzable-P between the transitional and impacted areas even though labile P_o in the former was about twice as great as in the latter.

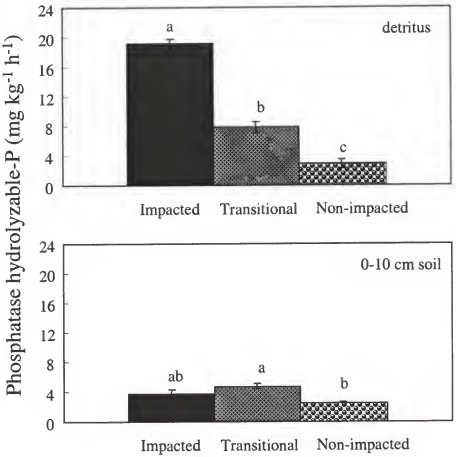

Fig.4.4 Organic P mineralized by alkaline phosphatase (2 units/g fresh sample) after 4-h incubation of detritus and soil sampled on March 1999 from WCA-2A. Error bars are standard error among three cores. Letters indicate least significant difference (LSD) at α=0.05.

Table 4.5. Rates of potentially mineralizable-P (PMP) measured after 10-d incubation of detritus/floc and soils at 40°C.

Site	Sampling date	Station	Depth	PMP
			cm	mg kg^{-1} d^{-1}
Blue Cypress Marsh†	Dec 1999	UIC	0-10	2.03±1.01
		(Non-impacted)	10-20	2.54±1.04
			20-30	3.05±0.48
			30-50	1.99±0.36
		Mean± SD		2.41
		NEI	0-10	2.28±0.79
		(Impacted)	10-20	1.26±0.47
			20-30	0.82±0.95
			30-50	0.32±0.63
		Mean± SD		1.17
WCA-2A‡	May 1998	U3	floc	5.31±1.49
		(Non-impacted)	0-5	4.40±0.75
			5-10	2.86±1.84
			10-20	0.88±0.57
			20-30	0.31±0.19
		Mean± SD		2.43
		F1	floc	19.44±4.35
		(Impacted)	0-5	10.55±7.90
			5-10	15.04±12.55
			10-20	9.69±12.28
			20-30	8.22±6.65
		Mean± SD		11.93
	Mar 1999	W-2	detritus	2.43±2.32
		(Impacted)	0-10	1.14±0.31
		W-5	detritus	4.55±1.14
		(Transitional)	0-10	1.02±1.29
		W-8	detritus	-1.56±11.08
		(Non-impacted)	0-10	1.14±1.02

† Each value represents mean ± standard deviation among 4 cores.

‡ Each value represents mean ± standard deviation among 3 cores.

Mineralization of Added Organic P

To gain more insights into the influence of substrate quality, mineralization of select P_o substrates was investigated for both impacted and non-impacted stations. Organic P substrates were incubated under aerobic or anaerobic conditions to determine the influence of redox or O_2 availability on P_o mineralization. Except for phytic acid, the hydrolysis of P_o substrates suggested no difference between aerobic and anaerobic incubations. Plot of net mineralization rate under aerobic versus anaerobic conditions yielded a slope of 0.88 with a correlation coefficient of 0.94 ($P<0.01$; n =38) (Fig. 4.5). Among the four P_o substrates, ATP was the most hydrolyzed while phytic acid was the least hydrolyzed. Approximately 70 and 60 % of added ATP and glucose-6-phosphate, respectively, were hydrolyzed for the 0-10 cm soils (Fig. 4.6). Glycerophosphate exhibited slow hydrolysis during a 4-h incubation in E2 soil, though substantial amounts (34-52%) were hydrolyzed between 24 and 360 h in both soils. For phytic acid, there was practically no hydrolysis that occurred in the non-impacted soil even after 48-h incubation. At the non-impacted station, there was a minimal release of P_i from phytic acid after 96 h, which gradually increased with incubation until at least 30% was hydrolyzed after 360 h. Hydrolysis of phytic acid at the P-impacted station, on the other hand, increased to 20% after 96 h and increased to about 60% after 360 h. Except for phytic acid, all P_o sources examined showed no difference in hydrolysis between incubations in impacted and non-impacted soils. There was a higher rate of hydrolysis ($P<0.10$; n=6) of phytic acid when incubated in impacted than non-impacted soils.

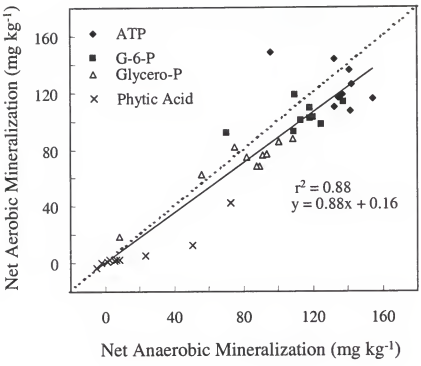

Fig. 4.5. Net mineralization of various P_o sources under anaerobic vs. aerobic incubations. The dashed line represents the relationship y = x. Organic P was added at 200 μg g^{-1}.

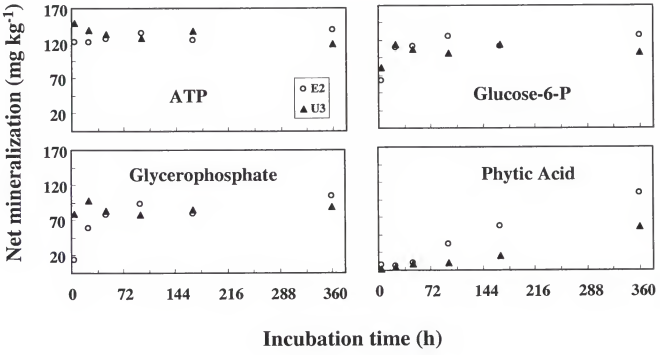

Fig. 4.6. Hydrolyses of various added organic P (200 μg g^{-1}) sources for 0-10 cm soils from P-impacted (E2) and non-impacted (U3) stations of WCA-2A. Except for the 360-h incubation, each point represents the average of aerobic and anaerobic incubations.

Influence of Litter Quality on P_o Mineralization

The contribution of plant residue to P_i after mineralization has been demonstrated in agricultural soils (Dalal, 1979; Chauhan et al., 1979) and wetlands (Puriveth, 1980; Findlay et al., 1990; Emery and Perry, 1996). However, there have been few studies pertaining to contribution of the type of plant residues on P_o mineralization in wetlands (Davis, 1991; Emery and Perry, 1996). The laboratory incubation provided potential mineralization rates for standing dead plants since mineralization of these materials may be moisture-limited under field conditions (Miao and DeBusk, 1999).

The initial SIPM was higher ($P<0.05$) when soils were incubated with cattail than sawgrass residues (Table 4.6). However, the final SIPM was not different between these plant residues. At the non-impacted station, the initial SIPM was higher ($P<0.01$) than the final SIPM regardless of added plant residues and incubation resulted to 35 and 18% reductions in SIPM with cattail and sawgrass, respectively. In contrast, the final SIPM was higher ($P<0.05$) than initial SIPM for soils from the impacted station with average C:P mass ratio of 611 and at the transistional station with average C:P mass ratio of 619 regardless of plant residue type. Both initial and final SIPM decreased with P enrichment. Final SIPM was positively correlated with APA at $P<0.10$ (Fig. 4.7).

The microbial biomass P after 30 d was different among stations and between plant residues at $P<0.01$. The transitional station had a higher microbial biomass P than either the impacted or non-impacted stations. On the one hand, microbial biomass P was higher with cattail than sawgrass as residues. There were significant differences in APA among stations and between plant residues (Table 4.7). Alkaline phosphatase activity was three times higher for the non-impacted than impacted stations. Moreover, APA was

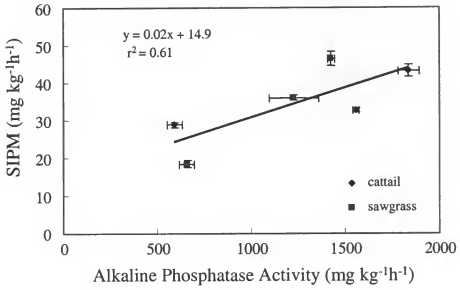

Fig. 4.7. Substrate-induced P mineralization (SIPM) after 30-d incubation for 0-10 cm soils with plant residues as a function of phosphomonoesterase activity (n = 3 stations × 2 residues).

higher (P<0.01) with cattail than sawgrass as residues. Phosphodiesterase activity was not significantly different between plant residues but different among stations at P<0.01. Phosphodiesterase activity decreased in the order: transitional>non-impacted>impacted. Among the microbiological and biochemical parameters, only APA demonstrated significant relation with SIPM. Alkaline phosphatase activity was positively correlated (P=0.05) with SIPM measured after 30-d incubation.

Table 4.6. Substrate-induced P mineralization (SIPM) of 0-10 cm soils before and after 30-d incubation with plant residues.

Station	Plant	P Added	Initial	Final
		mg P kg^{-1} dry soil		
Impacted	cattail	46	23.6 (0.3)	28.8 (0.7)
Transitional			25.7 (1.0)	32.7 (0.5)
Non-impacted			66.4 (4.2)	43.2 (1.6)
Mean±SD			38.6 ±24.2a	34.9 ±7.4a
Impacted	sawgrass	4	13.6 (0.3)	18.5 (0.9)
Transitional			22.9 (1.2)	36.1 (0.6)
Non-impacted			56.9 (0.8)	46.6 (1.9)
Mean±SD			31.1 ±22.8b	33.7 ±14.2a

Values in parentheses are standard errors among triplicates. Means with the same letters within a column are not significantly different at P<0.05. Cattail and sawgrass residues had 365 and 32 mg kg^{-1} TP, respectively. Average moisture content of the soil before residue addition was 92%.

Table 4.7. Influence of cattail (added at 46 mg kg^{-1} dry soil) and sawgrass (added as 4 mg kg^{-1} dry soil) on microbial and biochemical properties of 0-10 cm soils after a 30-d incubation.

	Station	cattail	sawgrass
Microbial biomass P (mg kg^{-1})	Impacted	32 (2)	24 (3)
	Transitional	95 (8)	60 (3)
	Non-impacted	20 (7)	15 (1)
	Mean±SD	49 ±40a	33 ±24b
Alkaline phosphatase activity (mg kg^{-1} h^{-1})	Impacted	593 (40)	659 (40)
	Transitional	1556 (16)	1229 (129)
	Non-impacted	1837 (55)	1426 (21)
	Mean±SD	1329 ±653a	1105 ±398b
Phosphodiesterase activity (mg kg^{-1} h^{-1})	Impacted	198 (13)	136 (4)
	Transitional	482 (68)	448 (56)
	Non-impacted	364 (24)	308 (24)
	Mean±SD	348 ±143a	297 ±157a

Means followed by the same letters within a row are not significantly different at $P<0.05$. Values in parentheses are standard errors among triplicates.

Discussion

Soils and detritus at stations distant from the water inflow are P-limited, and the availability of nutrients may be controlling P_o mineralization through enzymatic hydrolysis (Dalal, 1977; Stewart and Tiessen, 1987). Surficial detritus is a significant source of labile P_o (Reddy et al., 1993). The difference in pH stability between enzymes in WCA-2A and BCM can be attributed to the diversity of vegetation, microorganisms, and soil fauna (Frankenberger and Johanson, 1982). The difference in soil pH between BCM and WCA-2A resulted in differences in regulation of enzyme activity. Enzyme activity generally decreased with soil depth. This can be associated with decreases in substrate concentration (labile P_o), and in Ca^{2+} and Mg^{2+} concentrations with depth (Reddy et al., 1993). These metallic ions bind with phenolic and humic compounds, thereby liberating the enzymes from being complexed with these compounds (Wetzel, 1993).

Efficiency of microorganisms, repression-derepression of P_i, and labile P_o content can regulate phosphatase and phosphodiesterase activities. The activities of phosphatases are inhibited when P_i concentrations are high, while new enzymes are synthesized at low P_i concentrations (Cembella et al., 1984). In BCM, there was no difference in acid phosphatase activity between UIC and NEI because these stations had similar microbial efficiencies. In WCA-2A, the small population of microorganisms in the non-impacted station tended to be more effective by producing phosphatases in order to meet their metabolic needs. Thus, high microbial efficiency equates to high phosphatase production (Harrison, 1983). In addition, the low level of P_i at the non-impacted station allows unrestrained enzyme production. A number of studies have indicated that P_i inhibits

phosphatase production (Spiers and McGill, 1979; Newman et al., 2000; Kang and Freeman, 1999) while another study (Kobori and Taga, 1979b) did not observe such inhibition. Inhibition of enzyme activity is probably dependent on the amount of inhibitor present. Degobbis et al. (1986) and Taga and Kobori (1978) observed that alkaline phosphatase increased P_o hydrolysis in marine and ocean sediments having P_i concentrations below 4-6 μmole L^{-1}. Further study is needed to determine whether a higher phosphatase activity observed at the non-impacted than impacted stations of WCA-2A was the result of a response of the microbial biomass producing the enzyme or merely an enzyme activity activation caused by low inorganic P. In BCM, the higher phosphodiesterase activity at NEI than UIC coincided with the higher labile P_o at NEI than UIC.

Enzyme assays can be used to confirm results obtained from nuclear magnetic resonance (NMR) analysis. Relationship between APA and phosphodiesterase activity obtained for WCA-2A supports NMR studies that suggested the predominance of the monoester over diester form of P in alkali extracts of agricultural and pasture soils (Tate and Newman, 1982; Hawkes et al., 1984; Condron et al., 1985; Adams and Byrne, 1989; Condron et al., 1990). Using ^{31}P-NMR, Robinson et al. (1998) determined that 29-59% of the TP in wetland organic soils was in the monoester-P forms while only 6 to 10% of the TP was in the diester-P forms. Phosphodiesters that include phospholipids and some nucleic acids are more chemically labile than monoester-P; therefore, are easily degraded by phosphatases and microorganisms (Hinedi et al. 1988; Bedrock et al., 1994; Magid et al., 1996). At both sites, phosphodiesterase activity was not correlated with P_o mineralization; hence, a less labile substrate such as phosphomonoesters can regulate P_o

mineralization. However, in WCA-2A, regulation of enzyme activity by a repression-derepression mechanism and phenolics probably obscured the relationship sought for APA and P_o mineralization. A negative relationship obtained between APA and PMP in detritus/floc was probably due to the high levels of extractable-P_i. In wetlands, it has been demonstrated that alkaline phosphatase activity is deactivated under high P_i but is activated when P_i becomes limiting (Newman and Reddy, 1993; McLatchey and Reddy, 1998; Kang and Freeman, 1999). On the other hand, the non-significant relationship obtained between APA and PMP for soils was probably because of the formation of an enzyme-phenolic complex (Wetzel, 1993).

Microbial efficiencies and enzyme activities govern P_o mineralization in BCM. The PMP was not significantly different between UIC and NEI even though the labile P_o pool at UIC was higher than for NEI. This is because these stations had comparable microbial efficiencies and acid phosphatase activities. Unlike WCA-2A, the level of P_i in BCM does not inhibit enzyme activities; thus, PMP increased with acid phosphatase activity. In BCM, the positive correlations of acid phosphatase activity and microbial biomass P with PMP provided indirect evidence that microorganisms are the primary sources of acid phosphatases (Harrrison, 1982b).

Under non-limiting enzyme concentration, detritus and soils in WCA-2A demonstrated net mineralization with C:P mass ratios as high as 1375 while the critical C:P ratio is 300 (Stevenson, 1986). The higher phosphatase hydrolyzable-P in detritus than in 0-10 cm soils is likely due to the higher amounts of labile P_o in the detritus than in the 0-10 cm soil. In addition, the P_o in the detritus is less refractory than the 0-10 cm soil. In WCA-2A, Reddy et al. (1998) reported that residual or refractory P_o was 33% of TP

in the surface layer and increased to 70% of TP at lower depths. Meanwhile, the differences in phosphatase hydrolyzable-P of detritus among stations can be linked to the quality of litter. Sawgrass has a thick, waxy cuticle and several bands of lignified fibers on the leaf surface while cattail leaves have high water content and large inner spaces making them spongy (Miao and Sklar, 1998; Miao and DeBusk, 1999). Another reason is the presence of higher Ca and Mg concentrations in impacted/cattail than non-impacted/sawgrass areas (DeBusk et al., 1994). When enzymes are complexed with phenolic and humic compounds their activities are reduced (Wetzel, 1991; Chróst, 1991). However, Ca and Mg ions can bind with phenolic and humic compounds, thereby liberating the enzyme from the phenol- or humic-enzyme complex. For the 0-10 cm soil, the differences and similarities in phosphatase hydrolyzable-P_o among stations are dictated by the amount as well as the nature of labile P_o.

To gain more insights on the influence of the nature of P_o substrate, mineralization of selected P_o substrates were investigated at both impacted and non-impacted stations under both aerobic and anaerobic conditions. The influence of aeration on P_o mineralization is dependent upon nature of the organic substrate (Dalal, 1977). Suzumura and Kamatani (1995) observed that the hydrolysis of inositol hexaphosphate in coastal sediments was faster under anaerobic than aerobic conditions. Likewise, Reddy and Ivanoff (1991) observed faster hydrolysis of *p*-nitrophenyl phosphate on surface lake sediments under anaerobic than aerobic conditions, while there were no differences in hydrolysis due to aeration during incubation of glucose-6-phosphate and glycerophosphate. McLatchey and Reddy (1998), on the one hand, observed a threefold increase in RNA-P mineralization from anaerobic to aerobic soil conditions. Similarly,

Dick and Tabatabai (1978) reported higher P_o hydrolysis under aerobic than anaerobic conditions using glycerophosphate and other P_o substrates different from those used in this study. The effect of aeration on the hydrolysis of glycerophosphate was different between this study and that of Dick and Tabatabai (1978), probably due to the higher available P in agricultural soils than in soils used for this study. There can be inhibition of enzyme activities by P_i (Juma and Tabatabai, 1977; Speir and Ross, 1978).

Our study demonstrated that ATP and glucose-6-phosphate belong to the more labile P_o pools, glycerophosphate to the moderately labile pool, and phytic acid to the less labile P_o pool. Although the hydrolysis of phytic acid generally occurs at a low rate (Bowman and Cole, 1978b), its hydrolysis is enhanced under high-P soil conditions. Phytic acid has a high charge density and its adsorption to solid surfaces such as goethite, illite, or kaolinite protect it from microbial or enzyme attack (Anderson et al., 1974; Celi et al., 1999). The considerably higher microbial activity in WCA-2A probably yielded high phosphatase production, resulting in an appreciable hydrolysis of phytic acid.

Phosphorus is stored primarily through leaf production and aboveground detritus in wetland plants (Toth, 1987; 1988). Thus, the turnover of detrital materials can regulate nutrient availability in wetlands (Miao and DeBusk, 1999). The difference in initial SIPM between the residues was probably due to the difference in autolysis, with cattail releasing more P_i than sawgrass after being re-wetted (Martin and Cunningham, 1972; McLaughlin et al., 1988). It is also probable that the difference in initial SIPM may have been due to higher P added with cattail than sawgrass. Phosphorus added as cattail residues was more than ten times higher than when added as sawgrass residues. The excess P from cattail can serve as a nutrient source for microorganisms to degrade more

P_o. It is also likely that the higher cellulose content of cattail than sawgrass (Debusk and Reddy, 1998) caused decomposition to occur faster with cattail than with sawgrass. Further study is needed to determine whether the differences in P_o mineralization between cattail and sawgrass can be linked to differences in substrate quality or to an enhancement of the microbial activity after residue addition. Mineralized P_i appeared to be immobilized during the 30-d incubation of non-impacted soils that had an average C:P ratio of 1375 and immobilization was higher with cattail than sawgrass. The increase in SIPM after 30-d incubation of soils from impacted and transitional stations was due to the relatively low C:P ratios of these soils.

The topographic gradient from north to south of WCA-2A (DeBusk et al., 1994) caused a moisture gradient, with the optimum moisture content for microbial metabolism probably persisting at the transitional zone; hence, a higher microbial biomass was obtained in the transitional than impacted and non-impacted stations. Microbial activity in soil is optimal at –0.01 MPa and decreases when the soil becomes either waterlogged near zero water potential or dry at large negative water potentials (Paul and Clark, 1989). Microbial biomass P, on the one hand, was higher with cattail than sawgrass as residues. The type of surface detrital material can also influence the microbial biomass P pools in WCA-2A. A high microbial biomass is expected with plant residues having high cellulose content.

The difference in APA between cattail and sawgrass residues demonstrated that plant composition could influence enzyme activity (Gianfreda and Bollag, 1996). Repression-derepression mechanism, on the other hand, explained the observed differences among stations. It appeared that the addition of plant residues does not

influence phosphodiesterase activity; thus suggesting that phosphodiesters in WCA-2A are mainly of microbial origin (Cosgrove, 1977). Phosphodiesterase activity in soils can be derived from *Escherichia coli*, *Lactobacillus acidophilus*, *L. casei*, and *Micrococcus lysodeikticus* (Browman and Tabatabai, 1978).

The increase in APA with SIPM after residue addition suggests that the additional P from plant residues does not affect APA. It is also probable that the presence of residues increased substrate concentration, thereby allowing higher enzyme activity even in the presence of competitors like P_i (Newman and Reddy, 1992).

The quantity and quality of substrates, enzyme activity, and microbial efficiency are the most dominant factors influencing P_o mineralization in wetlands. However, enzyme activity itself is regulated by factors such as the presence of P_i, metallic activator or deactivator, moisture content, and type of vegetation. Phosphodiesterase activity does not regulate P_o mineralization in wetlands dominated by phosphomonoesters. The labile P_o content and quality of P_o substrate can influence phosphatase hydrolysable-P_o. The effect of aeration on P_o mineralization is dependent on the nature of P_o. Phytic acid can be considered as less labile; however, P enrichment can enhance its hydrolysis. The quality of litter added to soil can determine the rate of P_o mineralization.

Conclusions

Higher phosphatase activities in non-impacted than impacted areas indicated: (1) an increase in the efficiency of microorganisms utilizing the limited P supply, and (2) an uninhibited production of the enzyme because of the low amounts of P_i present. This study revealed that mineralization of P_o in wetlands was predominantly regulated by

phosphomonoesterase activities as compared to phosphodiesterase activities. Phosphatase hydrolyzable-P_o provided measures of the quantity as well as the quality of labile P_o required for mineralization. Results obtained for phosphatase hydrolyzable-P_o verified the higher rates of P_o mineralization for detritus than for soils in WCA-2A. For detritus, phosphatase hydrolyzable-P_o increased with P enrichment. The differences in phosphatase hydrolyzable-P_o among the stations also revealed differences in substrate quality with the easily degradable type in the P-impacted than in the non-impacted areas. In soils, higher phosphatase hydrolyzable-P_o for transitional than for non-impacted areas was coincident with high microbial biomass P and total labile P_o.

The effect of aeration or O_2 supply on P_o mineralization is dependent on the nature of P_o sources. Among the four P_o sources investigated, only phytic acid demonstrated differences in hydrolysis between aerobic and anaerobic conditions. This study revealed that ATP and glucose-6-phosphate could be grouped as the more labile P_o pool, glycerophosphate as the moderately labile pool, and phytic acid as the less labile P_o pool. Additional supply of P_i increased the mineralization of phytic acid, a more stable P_o source. Thus, wetland eutrophication can lead to the hydrolysis of even the more stable P_o sources.

The C:P mass ratio governed P_o mineralization in soils incorporated with plant residues. Addition of plant residues yielded increases in SIPM to soils from impacted and transitional areas that had relatively low C:P mass ratios. On the other hand, residue addition to soils from the non-impacted area that had a high C:P mass ratio resulted into a decrease in SIPM. Higher microbial biomass and APA for soils added with cattail than sawgrass residues explained the higher rate of SIPM with cattail than sawgrass residues.

Thus, residue addition alters microbial biomass P and APA that influenced P_o mineralization.

The activities of enzymes regulating P_o mineralization in wetlands are influenced by P enrichment, the quality of substrate, microbial efficiency, and labile P_o content. Organic P sources differ in their ease of hydrolysis; however, P loading to a wetland can increase the hydrolysis of even the more stable P_o sources. Plant residues added to a wetland can increase the microbial biomass P pool and phosphatase activity that regulate P_o mineralization.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Field and laboratory studies were conducted to examine mineralization rates of labile organic P in a freshwater wetland impacted with P, along with factors influencing such rate. The influences of inorganic electron acceptors, P-hydrolyzing enzymes, nature of the organic P substrate, and P enrichment on labile organic P mineralization were evaluated, with results reported in Chapters 2 to 4 to answer specific questions raised in Chapter 1. In this chapter, important findings of experimental studies described in Chapters 2 to 4 are briefly summarized according to the objectives set forth at the beginning of this dissertation.

1) Determine the effect of P loading and soil factors on the mineralization rate of labile organic P.

Higher rates obtained for substrate-induced P mineralization (SIPM) and potentially mineralizable-P (PMP) in detritus compared to surface soils were explained by higher microbial biomass P and labile P_o pools in detritus than in surface soils. Thus, microbial biomass and labile P_o content are the dominant factors regulating P_o mineralization in WCA-2A. Detritus and soils of WCA-2A had critical C:P_o mass ratios ranging from 300 to 550, and labile P_o mineralization is not affected by O_2 availability.

Inter-relationships among factors regulating P_o mineralization confounded the effects of P enrichment on P_o mineralization. For example, high amounts of inorganic P

in the detritus, inhibits the activity of alkaline phosphatase; high moisture content increases the amount of labile P_o, and the influence of redox on P_o mineralization is dependent on the nature of substrate.

Increases in SIPM for soils with decreases in P enrichment suggested increases in the efficiency of microorganisms utilizing the limited amount of P present. The decreases in SIPM with P enrichment for detritus, on the other hand, indicated inhibition of alkaline phosphatase activities by high levels of P_i. Potentially mineralizable-P can be a predictive tool in determining the turnover rates of various P pools. Relatively rapid turnover rates of 13 and 16 d were obtained for microbial biomass P and labile P_o pools. Restoration of an impacted wetland should take into consideration the turnover rates of P_o particularly the easily degradable or labile pools. When P loading to a wetland has been abated, the ecosystem can become P-sustainable for periods that can be dictated by the rates of P_o mineralization as well as P immobilization. Thus, the labile P_o pools are as equally important as the P_i pools in determining wetland eutrophication.

2) Determine the effect of inorganic electron acceptors on the rate of labile organic P mineralization

Net mineralization was less distinct in detritus under aerobic condition because P_i released through mineralization is immediately immobilized. Net mineralization occurred under nitrate-, sulfate-, and bicarbonate-reducing conditions but mineralization rates were not different among these conditions. For 0-10 and 10-30 cm soils, the type of electron acceptor does not affect PMP rates. For SIPM, the 0-10 and 10-30 cm soils demonstrated higher mineralization rates under aerobic than anaerobic conditions, while there was no difference in SIPM rates among electron acceptors for detritus.

Microbial biomass yielded positive correlations with SIPM under O_2- and SO_4^{-2}-reducing conditions but not under NO_3^- - and HCO_3^--reducing conditions. The study demonstrated that the microbial population does not respond to varying electron acceptor during long incubation such as 15 d. However, the microbial population exhibited increases in metabolic activities during short-term dry periods.

Wet and dry cycles involving short-term aerobic condition of wetlands can increase the mineralization of labile P_o. However, long dry periods such as 15 d would allow for precipitation or adsorption of P_i released after mineralization. The study demonstrated that in Ca-enriched ecosystems, P_o mineralization involving long-term incubation should account for precipitation of P_i released during mineralization. Precipitation, however, is negligible during short-term incubation periods such as 4 h.

3) Determine the influence of organic P substrate and extracellular enzymes on the rate of organic P mineralization

In WCA-2A, the higher alkaline phosphatase and phosphodiesterase activities at the non-impacted than impacted stations were due to higher microbial efficiency and activation of enzyme activity at low P_i concentrations. There was no significant difference in acid phosphatase activity between impacted and non-impacted stations in BCM because the stations evidenced similar microbial efficiency.

Phosphodiesterase activity did not regulate labile P_o mineralization for the freshwater wetlands evaluated. The influence of alkaline phosphatase activity on P_o mineralization, however is obscured by the presence of high levels of inorganic P, phenolic compounds, and reduced metals.

Four labile P_o substrates that were investigated can be grouped with respect to ease of hydrolysis: glucose-6-phosphate and ATP being more labile, glycerophosphate only moderately labile, and phytic acid as less labile. Among the P_o substrates evaluated, only the hydrolysis of phytic acid was affected by P enrichment. The hydrolysis of phytic acid was significantly higher for the P-impacted than non-impacted soils. Thus, wetland eutrophication can lead to the hydrolysis of even the more stable P_o sources.

Phosphatase hydrolyzable-P_o provided measures of the quantity as well as the quality of labile P_o required for mineralization. Results obtained for phosphatase hydrolyzable-P_o verified the rates of P_o mineralization for detritus being higher than for soils in WCA-2A. For detritus, phosphatase hydrolyzable-P_o increased with P enrichment. The differences in phosphatase hydrolyzable-P_o among the stations also revealed differences in substrate quality with the easily degradable type in the P-impacted than in the non-impacted areas. For soils, the higher phosphatase hydrolyzable-P_o in transitional than in non-impacted areas coincided with high microbial biomass P and total labile P_o.

4) Determine the influence of added residues on labile P_o mineralization

The C:P mass ratio governed P_o mineralization in soils incorporated with plant residues. Addition of plant residues yielded increases in SIPM to soils from impacted and transitional areas that had relatively low C:P mass ratios. On the other hand, residue addition to soils from the non-impacted area that had a high C:P mass ratio resulted into a decrease in SIPM. Higher microbial biomass and APA for soils added with cattail than sawgrass residues explained the higher rate of SIPM with cattail than sawgrass residues.

Thus, residue addition alters microbial biomass P and APA that influenced P_o mineralization.

As an overall conclusion, the mineralization of labile P_o can be an important biogeochemical pathway increasing the availability of P even after P loading to a wetland is abated. The wetland can be sustainable for periods as determined by rates of P_o mineralization. Phosphorus enrichment can lead to the hydrolysis of even the more stable P_o. Measurements of the microbial biomass P, labile P_o, SIPM, PMP, and phosphatase hydrolyzable-P_o are useful in assessing the contribution of P_o mineralization to the overall P cycling of an ecosystem.

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BIOGRAPHICAL SKETCH

Teresita Teneza Chua was born on April 22, 1961 to the inter-racial union of a hard-working and principled Chinese father and an equally hard-working Filipino mother. Family and friends fondly call her Tess. Being the second among a brood of seven children, she grew up learning how to deal with the whims of younger siblings and the idea of sharing. She earned her bachelor's degree in chemistry in 1981 at the University of Santo Tomas, a Catholic university in the Philippines. She spent six and a half years as a research assistant at the International Rice Research Institute (IRRI), Los Banos, Philippines. Much of her research interests in soils and agriculture stemmed from working with Dr. Roland J. Buresh, her immediate supervisor, who unselfishly introduced her to field and laboratory research on major soil nutrients – N, P, and S.

In June 1991, she married Celedonio Oña amidst the threat of an eruption of Mt. Pinatubo. Immediately after this transition from single to married life, she decided to move to the United States to pursue her dire interest in soil chemistry. Her master's work entitled "Contribution of vesicular-arbuscular mycorrhizal hyphae to phosphorus acquisition of *Andropogon Gerardii*" was under the advisorship of Dr. A. Paul Schwab at Kansas State University. She went back to the Philippines right after she finished her M.S. degree and from thereon decided to pursue her Ph. D. with Dr. K. Ramesh Reddy at the University of Florida. After her first year at UF, she gave birth to Cheltea who has been her inspiration to this major undertaking.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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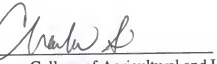

Dean, College of Agricultural and Life Sciences

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